In vitro Screening and Biochemical Studies on Salt- tolerance in *Trifolium alexandrinum*

THESIS

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By

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Under the supervision of

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Dedicated to
Yaggesh,
"My Candle in the Wind"

ICAR

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To,

The Vice Chancellor
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Sub: Submission of Ph.D. Thesis.

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Sir,

I am forwarding herewith the thesis entitled "*In vitro* Screening and Biochemical Studies on Salt-tolerance in *Trifolium alexandrinum* " by Mr. Kuldip Dwivedi for the degree of Doctor of Philosophy (Botany), Bundelkhand University, Jhansi. The work has been carried out at Indian Grassland and Fodder Research Institute, Jhansi under the supervision of Dr. D. R. Malaviya.

Thanking you

Yours faithfully,

(K. A. Singh)

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CERTIFICATE

It is certified that this thesis entitled "*In vitro* Screening and Biochemical Studies on Salt-tolerance in *Trifolium alexandrinum*" is an original piece of work done by Mr. Kuldip Dwivedi under my supervision and guidance for the degree of Doctor of Philosophy (Botany), Bundelkhand University, Jhansi.

I further certify that:

- It embodies the original work of candidate himself.
- It is up to the required standard both in respect of its contents and literary presentation for being referred to the examiners.
- The candidate has worked under me for the required period at Indian Grassland and Fodder Research Institute Jhansi.
- The candidate has put in the required attendance in the department.

19/9/06

D. R. Malaviya
(Supervisor)

DECLARATION

I hereby declare that the thesis entitled "*In vitro* Screening and Biochemical Studies on Salt-tolerance in *Trifolium alexandrinum*" being submitted for the degree of Doctor of Philosophy (Botany), Bundelkhand University, Jhansi (UP) is an original piece of research work done by me under the supervision of Dr. D. R. Malaviya, IGFRI, Jhansi and to the best of my knowledge, any part or whole of this thesis has not been submitted for a degree or any other qualification of any University or examining body in India / elsewhere.

Kuldip Dwivedi

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Kuldip Dwivedi

(Kuldip Dwivedi)

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INTRODUCTION

INTRODUCTION

Soil salinity is one of the major abiotic stresses, which significantly affects crop productivity throughout the world (San Pietro, 1982). An FAO study in 1989 estimated that up to 7% of the world's land area is salt affected (Szabolcs, 1994). A large area is getting salt affected due to recent agricultural practices. The total area of human induced degraded soils was assessed at 1964 m ha, which does not include land degraded by ancient civilizations or by colonial expansion, nor the land that is naturally barren, is an alarmingly high figure (Ghassemi et al., 1995). In arid and semi arid regions, the ground water is often rich in soluble salts. The water tables rises when the land is cleared of perennial vegetation, or irrigation schemes are installed, and once the ground water rises within 2 meters of soil surface, both root uptake and evaporation results in the salt concentration rising and the soil becomes saline or sodic. If the salt concentration is high enough to lower the water potential appreciably (0.5 – 1.0 bar), the soil is said to be salt affected (Levitt, 1980).

In pursuit of improving the agricultural production to meet the growing demands for food, fuel and fodder of an ever-increasing population, several major and minor irrigation projects have been launched in India since independence under various five-year plans. Though initially, the projects proved to be beneficial to the farmers, water logging and subsequent secondary salinization gradually started developing. Extensive seepage from canals, distributaries, channels and reckless wastage of water in drains has obstructed the natural drainage in many cases. All these processes have led to the development of waterlogged conditions and subsequent salinization and/or alkalization. According to estimates of FAO and UNESCO, as much as half of the existing irrigation systems of the world are under the affects of secondary salinity, sodicity or water logging (Sczablocs, 1994).

The salt stress may have primary and secondary effects. Primary salt effects include metabolic disturbances and inhibition of growth and development, while secondary salt effects include nutrient deficiency and osmotic dehydration. In general, majority of the existing cultivars give low yield in saline soil. The major efforts to circumvent salinity in the past have been directed towards soil reclamation and water desalinization practices that are increasingly expensive. Thus, for improving biomass production and yield in salt affected soils, emphasis has generally been on improving the intrinsic salt tolerance of the

plants. Although several mechanical and chemical methods have been devised to reclaim the salt- affected soils, they are expensive and are not readily feasible. Hence, identification of plant species/varieties that can tolerate high salt levels is presently being considered important for utilization of these soils. The efforts must, therefore, coincide with measures to improve the salt resistance of crops through genetic modification. The rate at which the salinity problem is increasing, it is important to have salt resistant varieties of economically important crops.

In some species the diversity for salt resistance among cultivars is extensive and conventional breeding techniques are being used to improve the resistance in related genotypes/varieties. In species with little diversity for salt resistance, promising approaches would be either to use variation existing in wild relatives or to use tissue culture techniques for selection of mutation for salt resistance (Tal, 1983). Further, most of the earlier studies on salt tolerance have been restricted to evaluation of a few genotypes under that condition, whereas it is being realized that variation existing at genotypic level as well as the variation at intra-genotypic level in the crops having heterozygous background could also be exploited. Intra and inter genotypic variation for salt tolerance has also been reported in Lucerne by Al- Khatib et al. (1994) additionally intra-cultivar variations have been identified in Lucerne and *T. repens* allowing selection programme to be undertaken (Rogers and Noble, 1990).

Salt-tolerance is a complex, multigenic trait and is often a composite response of the integrated biological system. Hence, bio-chemical/molecular markers are considered to be good indicators and are being used for fast identification of salt tolerant lines and/or plants. The use of isozymes as markers has also been an approach of considerable value apart from being used as markers for the population and evolutionary studies.

For screening of the lines under salt stress, the common practice has been to evaluate the lines either in pot culture or under field conditions. In the recent past some work has been done on screening of the lines *in vitro* condition. Screening *in vitro* condition provides uniform environment to all genotypes, and thus could be more reliable. Moreover, the study on biochemical changes on the plants growing *in vitro* under salt stress also provide better opportunities to compare the results with the plants growing under normal conditions *in vitro.* Foolad and Jones (1993) advocated that salinity under field conditions is complicated by the heterogeneity in salt concentrations at different depths in the soil, time and space. Hence, evaluation of germplasm should be done after providing best

possible uniform condition. For over a decade cell culture has been advocated in selection programmes for salt tolerance. Selecting cultured cells for survival at high NaCl potentially offers a fast means for generating, evaluating and selecting genotypes with superior salt tolerance. Hasegawa et al. (1995) advocated improvement of salt tolerance in plants by application of tissue culture to obtain salt tolerant plants and for identification and characterization of cellular determinants of salt tolerance.

India with huge cattle population faces nearly 40% fodder deficit. This amounts to low productivity of the milking animals. It has also been realized that in last few decades area under forage cultivation has not increased due to farmer's preference for cash crops. Thus, the plausible alternative is to search for possibilities of growing forages in non-traditional or non-arable lands such as saline/sodic lands.

T. alexandrinum (Egyptian clover or Berseem), one of the most important Rabi fodder crop, is reported to possess tolerance for salinity (Raheja, 1966) and thus could be used for reclamation of saline soils. In spite of slow reclamation of soil by Berseem cultivation, it has been advocated as an economical way to bring the land into use. If we can develop varieties or screen salt tolerant lines from the existing germplasm it could prove beneficial in the reclamation process in addition to increasing area under Berseem cultivaion.

Berseem (Egyptian clover) is thought to have originated in Asia minor, from where it was brought to Egypt through Palestine and Syria. It was probably the earliest forage crop grown in Egypt. In India it was introduced into Sindh state (now in Pakistan) in 1904 for the first time. It is an annual plant (30-90cm tall) with hollow and very succulent stem. Berseem is very palatable and nutritious fodder, containing 15-21% crude protein, 1% Ca, 22-26% crude fibre and 12% ash (Patil and Mistry, 1960). Looking into the increasing demand for fodder and shrinking area under forage cultivation the problem soils are the only area where from forage production can be given a boost. Among the various forages Berseem is cultivated in about 2 m ha area. Considering its high production potential and wide adaptability to diverse growing conditions, the crops adaptations to saline/sodic conditions needs to be evaluated. Despite its great economic importance less efforts has been made to improve its salt tolerance. Thus, the development of rapid screening method and enhancement of salt tolerance through application of tissue culture techniques can be of great value.

The proposed work has been envisaged to screen the Berseem accessions for existence of inter and intra-genotypic variation for salt tolerance. The biochemical studies on the plants growing under stress condition may also reveal the biochemical attributes linked to salt tolerance. The *in vitro* callusing study may be helpful in inducing salt tolerance and developing cell lines having tolerance to varying levels of salt. The tolerant plant thus identified can also be taken directly to field condition. Hence, the study has been planned with following objectives:

1. *In vitro* screening of *Trifolium alexandrinum* lines for varying levels of salt concentration.
2. Studies on biochemical attributes related to differential response under control and stress conditions.
3. Studies on salinity tolerance under *in vitro* culture conditions at different salt concentrations.

REVIEW
OF
LITERATURE

REVIEW

A. Extent of agricultural salt problem

A.1. Global Scale

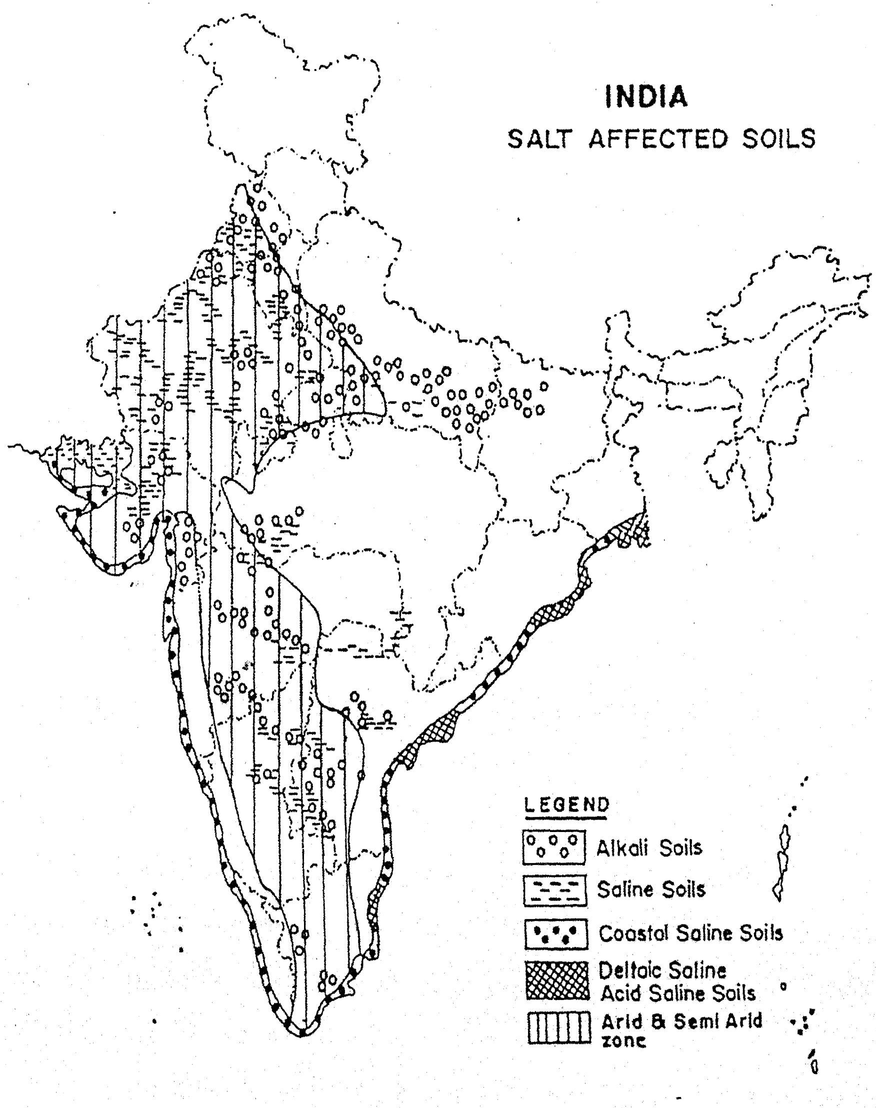
Most of the waters in the hydrosphere are salty and much of the fresh water is frozen. The world's land surface occupies about 13.2 x 10^9 ha, of which no more than 7 x 10^9 ha is arable and only 1.5 x 10^9 ha of which are cultivated (Massoud, 1981). Of the cultivated land, about 0.34 x 10^9 ha (23%) are saline. Another 0.56 x 10^9 ha (37%) is sodic (Szablocs, 1989). Thus, saline and sodic soils cover about 10% of the total arable land and exist in over 100 countries. Salt–affected soils are not limited to semi-arid to arid regions only. In several other regions, the climate and mobility of salts produce saline water and soil seasonally.

Salt affected soils occupy extensive area and occur globally. The extent of land that is salt-affected has long been uncertain and remains so. Based on the FAO/UNESCO Soil map of the world, the extent of salt-affected soils has been estimated by Massoud et al., (1981) to be 901.4 m ha. Flowers et al., (1986) estimated the area between 340 to 950 m ha. There is however, a great deal of variation in the distribution of saline land over the world's surface so that the regional impact of salinity is much more serious; for example about 26% of the 16 x 10^6 ha of cultivated land in Pakistan was salt-affected in 1978-79 (Ahmed, 1990). The total area of salt-affected land in Australia has considerable potential for expansion because it is commonly caused by rising water table as native vegetation is cleared for agriculture and often irrigated (Mc Willian, 1986). The impact of salinity is heightened by its connection with irrigation. Szabolcs (1989) quotes from FAO and UNESCO estimates that as much as half of all the existing irrigation systems of the world are more or less under the influence of secondary salinization, alkalization and water – logging. About 10 x 10^6 ha of irrigated land in Africa, is thought to be abandoned each year because of the adverse effects of secondary salinization and alkalization.

A.2. Status of saline soils in India

The area under salt affected soils in India was estimated to be 7 million hectares by Abrol and Bhumbla (1971). However, during the last decade several agencies have given divergent estimates e.g. National Commission on Agriculture (7.6 m ha), National Remote Sensing Agency (3.9 m ha), National Wasteland Development Board (1.5 m ha), National Bureau of Soil Survey and Land use Planning (6.2 m ha). The data from various

Fig.1. Distribution of salt affected soils in India. (Source: Gupta and Gupta, 1997)



sources were critically evaluated at Central Soil Salinity Research Institute, Karnal (1996) and figure has now been modified to 7.4 million ha.

The major states affected by soil salinity are Gujarat having 16.49 lakh ha of area affected by salinity, followed by Rajasthan 11.8 lakh ha, Uttar Pradesh 9.50 lakh ha and West Bengal 8.20 lakh ha. Saline and alkali soils are termed as usar in Indian terminology (usar has been derived from the Sanskrit word "Ustra" which means infertile soils). Salt-affected soils are estimated to about 12 million hectares of land. Singh (1992) estimated 34.3 x 106 hectares of land to be affected by salinity and alkalinity. These soils are locally known by different names in different parts of the country, viz "Reh", "Rahala, Usar in Uttar Pradesh; Thur, Kallar, Rakkar, Bara and Beri in Punjab and Haryana; Khar and Luna in Gujarat Chopan and Karl in the Deccan; Choudu and Uppu in Andhra Pradesh and Kari in Kerala. Salt effected soils occur in India in association with the normal zonal soils of the arid and semi- arid regions. These soils commonly occur in the following four major tracts.

1. The semi–arid Indo –Gangetic alluvial tracts (mainly in Punjab, Haryana, U.P and parts of Bihar).
2. The arid tracts of Rajasthan and Gujarat.
3. The arid and semi –arid tracts of southern states
4. The coastal alluvium.

B. Causes of Saline/alkaline soil formation

Salinity is the concentration of dissolved mineral salts present in water and soils on a unit volume on weight basis. The major solutes comprising dissolved mineral salts are the cations Na, Ca, Mg, K and S the anions Cl, SO_4, HCO_3 and NO_3.

Arid climates, Influence of sea water, Irrigation with saline water, High water table, Imperviousness of sub-soil, Imperfect/ defective drainage, Nature of parent materials, Volcanic effects, Use of basic fertilizers and Formation of plough soil are some of the factors responsible for the formation of saline – alkali soils.

The soluble ionic constituents of salt-effected soils are derived mainly from primary materials and to smaller extent from atmospheric sources through intermediary biological activity. The excess of salts found in soils is derived mostly from weathering and biological activity in other locations. Ocean water may be the immediate source of salts in coastal areas and in uplifted marine sediments. Commonly the salts are gradually accumulated through underground or surface movement of water from location of higher

elevation to those of lower elevations, followed by evaporation of water. In the United States, operation of this process on a large scale has produced the Great Salt Lake in Utah, and a large area of strongly saline soils around. Since the areas at low elevations in which salts may accumulate are generally the most adapted to crop production from the standing point of topography and ease of irrigation, the problems of soils salinity are of major importance in highly developed agriculture in dry and semi-dry regions. Irrigation waters also contain soluble salts and in some instances as much as 20 metric tonnes of salts are added in this way per hectares annually.

C. Crop / Varieties suitable for Saline / Sodic conditions

Plants have been categorized as halophytes or glycophytes depending upon their responses to salinity. Even this distribution is not absolute because species range from highly tolerant to very sensitive. The tolerance of individual plant species and varieties to soil salinity increases with their capacity to adjust to relatively high internal solute suctions and decreases with their sensitivity to this adjustment. Plants native to a saline environment have both a marked capacity for adjustment and limited sensitivity. Crop plants have a lesser but still considerable capacity for upward adjustment of internal solute suctions, but they are more sensitive to the adjustment.

A number of classifications of crops as regards tolerance to salinity have been developed in different parts of the world. Various criteria for appraising the tolerance of plants to soil salinity may be employed. Mass (1993) quantified the crop response to salinity by plotting relative growth or yield as a continuous function of increasingly higher levels of soil salinity.

D. Forage Availability / Shortfalls

Estimates show that only 40% of the feed and fodder requirement can be met through all the forage sources available in India. Only 4.4% area is available for the cultivation of fodder corps. With the ever-increasing food demands, the opportunities of increasing area under fodder crops are virtually not possible. To overcome this deficiency, the under-utilized and unutilized land resources including salt–affected lands should be exploited for forage production. Salt affected lands are otherwise unfit for crop production. Conventional technologies for reclamation of alkali and saline soils are very costly and beyond the reach of marginal and average farmers thus call for alternative land use. Forage gasses like Karnal grass (*Leptochloa fusca L.*), Rhodes grass (*Chloris gayana*), Gatton Panic (*Panicum maximum*), Bermuda grass (*Cynodon dactylon*) and Para grass

Table 1. Crops and their threshold salinity level of tolerance

Crop	Threshold a salinity level (EC, dS/m)
Tolerant crops	
Sugar beet *(Beta vulgaris)*	7.0
Cotton (*Gossypium hirsutum*)	7.7
Barley (*Hordeum vulgare*)	8.0
Bermuda grass (*Cynodon dactylon*)	6.9
Tall wheat grass (*Agropyron longatum*)	7.5
Moderately tolerant crops	
Cowpea (*Vigna unguiculata*)	4.9
Soybean (*Glycine max*)	5.0
Perennial rye grass (*Lolium perenne*)	5.6
Wheat (*Triticum aestivum*)	6.0
Durum wheat (*T. turgidum*)	5.7
Sorghum (*Sorghum bicolor*)	6.8
Moderately sensitive Crop	
Berseem clover (*Trifolium alexandrinum*)	1.5
White clover (*T. repens)*	1.5
Red clover (*T. pratense*)	1.5
Alfalfa (*Medicago sativa*)	2.0
Corn (*Zea mays*)	1.7
Rice (*Oryza sativa*)	3.0
Tomato (*Lycopersicon sp.*)	2.5
Sugarcane (*Saccharum officinarum*)	1.7
Lettuce (*Lactuca sativa*)	1.3
Sensitive crops	
Bean (*Phaseolus vulgaris*)	1.0
Carrot (*Daucus carota*)	1.0
Onion (*Allium cepa*)	1.2
Orange (*Citrus sinensis*)	1.7
Peach *(Prunus persica*)	1.7
Plum (*P. domestica*)	1.5
Apricot *(P. armeniaca*)	1.6

a = Maximum salinity level at which a crop has no yield.

(Source: D. P. Singh, 2002).

(*Bracharia mutica*) have been found tolerant to saline soils. Most of the grasses bring out improvement in the physical, chemical and biological properties of alkali and saline soils but karnal grass has been found to be the best. Growing of shaftal (*Trifolium resupinatum* L.) as a forage crop, in winter (Rabi) season has been found to be more profitable than growing wheat (*Triticum aestivum*) (Sharma et al., 1983). Grasses and forages, because of their thick canopy, form a mat on the soil, which therefore receives less heat and dries up less (FAO/UNESCO, 1973). Since grasses transpire a great deal, they help in lowering the water table. Further, the perennials enrich the soil with organic substances through their extensive root system, which improves the soil structure and hydro-physical properties of the soil profile. Inclusion of perennial plants and leguminous forage crops in a crop rotation, also helps to intensify the biological activity of soil, increases the nitrogen supply and build up the soil fertility.

Amongst the forage crops, winter–season (Rabi) crops seem to be relatively more tolerant than rainy–season (kharif) crops. Sorghum, Bajra (pearl millet) and dhaincha (*Sesbania*) are moderately tolerant forage corps. The entire Rabi forage crop are moderately tolerant, however Oat and shaftal are more tolerant than Berseem (Ashok Kumar, 1987).

E. Berseem Crop

Trifolium alexandrinum (Egyptian clover) is popularly known as Berseem. The name Berseem is derived from Arabic name "Bersym" or Berzum (Shukla and Patil 1985). The crop is believed to be indigenous to Egypt (Narayanan and Dabadghao, 1972) but has domesticated well in India. In, India it was introduced into Sindh state (now in Pakistan) in 1904 for the first time (Singh, 1993).

Berseem is one of the true clovers. It is an annual plant (30 to 90 cm) with hollow and very succulent stem. The roots do not extend far into the soil (about 30 cm deep) and are of medium size, being long and tapering, branched, fibrous and single or clustered. The stems are decumbent, ascending somewhat with prominent transverse rings, diffuse, fistulous, glabrous at the base and increasingly pilose above. The leaves are trifoliate, petiolate and oblong lanceolate to oblong elliptical leaflets, pubescent on both sides. Berseem has an inflorescence of dense head terminating the stem and branches. The seeds are sub orbicular to ovoid and 2 mm long. Seed coats are first dull, becoming shiny on exposure, glabrous and yellow tinged with brown in the regions of helium and the chalaza.

Berseem is the most important winter season legume cultivated in an area of around 2 million hectares in India. The significance of this forage species lies in the development of milk industry. It appeared to behave as a most potent milk multiplier in the lactating buffaloes, Sahiwal cows and cross breed cattle's as compared to other forage crops alone on in combination. Of the two Egyptian biotypes of Berseem 'Mescavi and Fahli' introduced in India during 1903, the former proved to be highly adaptable and productive as fodder crop for wide scale cultivation. Most of the present day cultivars are derivative of Mescavi. The merit of these cultivars lies in their multicut nature (4-8 cuts), long duration of fodder availability (November to April) and very high green fodder yield (85 t/ha), better quality (20% crude protein), high digestibility (up to 65%) and palatability. The phenomenal success of Berseem in India is also due to its high nitrogen fixing ability resulting in substantial improvement in soil fertility. Considering it's high production potential and wide adaptation in the tropical and sub tropical zone of the country, attention is being paid for it's further genetic improvement and it's adaptability to different biotic and a biotic stress conditions. According to Raheja (1966), *T. alexandrinum*, possesses moderate tolerance for salinity and can be used for the reclamation of saline soils. Although reclamation of soil by growing this crop is slow, it has been advocated as the most economical way to bring the land into use. A report regarding resistance to saline soils was published from India giving indications of tolerance in some lines of Egyptian clover. A study from Egypt (Mitkees et al., 1972) indicated a local variety 'Wafir' to be a resistant to saline soils. The use of natural and wild populations growing on saline sites may provide the required genetic material for the development of salt tolerance in the cultivars of *T. alexandrinum*.

F. Salt tolerance of Egyptian clover

T. alexandrinum is reported to possess tolerance for salinity (Raheja 1966) and thus could be used for the reclamation of saline soils. This species has been considered to be moderately salt tolerant (Winter and Lauchli, 1982). Comparative study of salt response in *T. alexandrinum* and *T. pratense* grown in culture solution at salinity levels of 50, 100, 150 and 200 mM NaCl showed that *T. alexandrinum* survived at all treatments whereas *T. pratense* showed a low survival potential at salt treatments of 100 mM NaCl or higher and the dry weight production of all plant parts was considerably affected at moderate salt levels. The distribution and contents of K^+, Na^+ and Cl^- in both the species indicate that *T. pratense* translocates Na^+ and Cl^- linearly into stems and leaves, whereas low foliar Na^+

and Cl^- contents in *T. alexandrinum* suggest some mechanisms that control the ion distribution in the different plant parts. The most striking difference in ion distribution between the two species is in the leaves, where K^+ content at all salt regimes was much higher in *T. pratense* than in *T. alexandrinum.* Despite lower Na^+: K^+ ratio in the leaves, leaf dry matter production was much lower (with respect to the controls) in *T. pratense* than in *T. alexandrinum*, may be the reason for it better performance at moderate salt stress. Considerable intra-ecotype variation exists for salinity and alkalinity tolerance in *Trifolium alexandrinum* L (Chandramohan, 2001). Amongst crop plants (Lessani and Marschner 1978; Greenway and Munns, 1980) it would range between the moderately salt tolerant beans and maize. In *T. alexandrinum* leaf production is less reduced by moderate salinity than in stem production, foliar salt levels are also low under moderate salinity, which seems to account for its success as a forage crop under slightly saline conditions.

Ashraf (1989) evaluated the effects of salinity on some cultivars of Berseem (*Trifolium alexandrinum)* and concluded that dry weight of plants harvested 5 weeks after the start of salt treatment, just before flowering, decreased with increasing salinity except in the 3 tolerant cultivars. The most tolerant cultivars 'Faisalabad late' had higher shoot and root Na^+ and Cl^- contents than the other cultivars at all salinity levels. There was no consistent correlation between biomass and ion content.

Studies have shown significant interspecific and intraspecific variation among Lucerne and clover species for salt tolerance. Intracultivar variations have been identified in Lucerne and *T. repens* allowing selection programmes to be undertaken. (Rogers and Noble, 1990). Noble and Shannon (1990) reported that control of Cl^- uptake in Lucerne (*Medicago sativa*) and white clover (*Trifolium repense*) was found to be effective criterion for its *in vitro* selection for salt tolerance.

Rogers et al., (1997) proposed further that it is possible to increase levels of salt tolerance in white clover by selecting for low shoot Cl^- concentrations under early stages of exposure to NaCl (i.e. day 4 or 5). Variation in salt tolerance and ion accumulation among subterranean clover cultivars was evaluated by Shannon and Noble, (1995). They advocated that productivity of clover under saline conditions requires high growth potential and low reduction in yield with increasing salinity. High productivity under saline stress was positively correlated with restricted Na^+ uptake in the shoot and the maintenance of high K^+ / Na^+ ratio. The use of natural populations of *T. repens* growing

on saline and non-saline sites may provide material for the development of salt tolerance in cultivars, plants from salt marsh sites showed high or very high salt tolerance with relatively vigorous root growth in 150-200 mM NaCl Shakur et al. (1988). They advocated that marintime populations might provide materials for the development of salt tolerance in cultivars.

G. Mechanism of Salt tolerance

Salinity exerts complex effects on the plants as a result of ionic, osmotic and nutritional interactions, although the exact physiological mechanism of salt stress is unknown. Salt tolerance often depends on the anatomical and physiological complexity of the organized plant. This fact makes it difficult to find ways to increase salt tolerance to large degrees. Studies on the mechanisms that underlie salt toxicity and salt tolerance of plants have revealed the involvement of many aspects of cellular, tissue and whole plant biology. Investigators have demonstrated salt tolerance mechanism based on factors such as ion accumulation (Rush and Epstein, 1976, 1981; Tal and Shannon, 1983), ion exclusion (Abel, 1969; Noble et al., 1984), compatible solute production (Grumet and Hanson, 1986.) pollen sterility (Akbar and Yabuno, 1977). It has been suggested that several of these factors can be selected and combined in a reengineered individual, a process referred to as pyramiding characters (Pasternak, 1987; Yeo and Flowers, 1983).

G.1. Ion Selectivity:

The most basic underlying attribute of salt tolerance is the maintenance of ion homeostasis in the cytoplasm. The transport of ions in and out of the cytoplasm is one major factor that regulates ion homeostasis. Ion transport into the cytoplasm is determined by an electrochemical gradient across cell membranes (Plasma membrane, tonoplast), carrier protein and ion ports. Under salt stress a plant must absorb nutrients and restrict the uptake of toxic ions at lower water potential than usual. Plants that limit uptake of toxic ions and maintain normal range of nutrient ion could be more salt tolerant than those that do no restrict ion accumulation and lose nutrient balance. Selective ion uptake mechanisms capable of discrimination between chemically similar ions such as Na^+ and K^+ could have adaptive value. The mechanisms responsible for ion discrimination probably are located in the membranes of tissues and various organelles throughout the plant (Bliss et al., 1984; Kupier 1968). Breeding for efficient nutrient uptake on low ion accumulation under salt stress may be among the simplest ways to improve salt tolerance in sensitive varieties of some species. This also may be accomplished by finding tolerance

to the toxicity of a specific ion associated with salt stress. Among the plant species, mangroves have the most efficient system of restricting salt uptake through the development of a passive root membrane filtration system. The gray mangrove (*Avicennia marina*) can exclude 90% of the salt in the medium surrounding its roots (Burchett et al., 1984). It has maximal growth at 25% seawater. Other mangrove species can survive salt concentrations two or three times that of seawater (Clough, 1984). The system in mangroves is unique and unfortunately, has not been reported in other species; most of the crop species limit salt uptake into the transpiration system to some degree through membrane-mediated compartmentation in organelle (Vacuoles) or tissue (Shannon 1997). Some plant species may be able to rid themselves of toxic ions through different physiological mechanisms such as ion sequestering organelles (Salt glands) or by storing salt in the roots, old leaves, petioles, stones or tracheids. Selective ion transport differences among species and varieties are the result of specific gene differences.

G.2. Ion Accumulation:

Halophytes take up high concentrations of ions as an adaptation mechanism to saline environments (Flower et al., 1977). The accumulation of salt in the plants or it's excretion onto leaf surfaces, in specialized organs such as salt glands and bladders is believed to reduce the requirements for increased wall extensibility that might otherwise be required to maintain positive growth and turgor at low soil water potentials. The wild tomato species (*Lycopersicon chessmanii*) is considered to be more salt tolerant than the cultivated species due to it's capacity to accumulate ion (Rush and Epstein, 1981b) and the salt tolerant "Edkawy" tomato also accumulates higher concentrations of Na^+ in leaf tissues than the more sensitive cultivar of *Lycopersicon esculentus* (Hashim et al., 1986). In both glycophytes and halophytes, salt may accumulate preferentially in vacuoles, interstitial compartments, stems or older leaves. The physical and genetic factors that influence ion compartmentation within plants are almost unknown. Few crop species e.g. Sugar beet, are halophytic and it is probably difficult to transfer halophytism into glycophytic crop species.

G.3. Organic Solute Accumulation:

Solute that is compatible with the cytoplasm is accumulated by all groups of organisms. Plants accumulate these solutes in response to low water availability, low temperature and salinity (Bohnert et al., 1995). Although they play a crucial role in higher plants growing under stress condition, its relative contributions varies among species, among cultivars and even between different compartments within the same plant. The compatible

osmolytes generally found in higher plants are low molecular weight sugars, organic acids, polyols and nitrogen containing compounds such as amino acids, amides, ectoine (1,4, 5-6-tetrahydroip-2-methyl-4 carboxylpyrimidine), proteins and quaternary ammonium compounds.

According to Cram (1976) sugars contribute up to 50% of the total osmotic potential in glycophytes subject to saline conditions. The accumulation of soluble carbohydrates in plants has been widely reported as a response to salinity on drought, despite a significant decrease in carbohydrate concentrations in response to salt stress. According to Rathert, (1985) increase in sucrose and decrease in starch was observed in response to salinity in *Glycine max*. Ashraf and Tufail (1995) determined the total soluble sugars content in five sunflower accessions differing in salt tolerance. They found that although sugar content increased significantly in all five lines with increasing salt in the growth medium, the salt tolerant lines had generally greater soluble sugar than the salt sensitive ones. Trehalose, a disaccharide, accumulates in many organisms under various a biotic stresses and has been reported to be both an osmolyte and an osmoprotectant (Crowe et al., 1984; Hounsa et al., 1998). It protects membranes and a protein in cells exposed to stresses that cause water deficit (Garcia et al., 1997; Goddijn et al., 1999) and reduces aggregation of denatured proteins (Singer et al., 1998). The role of sugars in adaptation of plants to salinity needs further investigation to finally conclude their association with salt tolerance. However, this does not rule out a significant role of soluble sugars in salt tolerance or their potential role as an indicator for salt tolerance in further breeding programmes.

Salt stress proteins, which accumulate only due to salt stress may provide a storage form of nitrogen that is re-utilized when stress is over (Singh, 1987) and may play a role in osmotic adjustment. Proteins may be synthesized *de nova* in response to salt stress or may be present constitutively at low concentrations and increase when plants are exposed to salt stress (Pareek et al., 1997). A 26Kda protein 'osmotin' was characterized to be salt induced protein in tobacco (Singh et al., 1987). Two 26Kda polypeptides, not immunologically related to osmotin, identified as germin, were found to increase in response to slat stress in barley (Hurkman et al., 1991). Uma et al., (1995) found 54Kda and 23-24Kda proteins responsible for salt or drought tolerance in finger millet (*Eleusine coracana*). Yamada et al., (2002) while investigating the mechanisms of salt tolerance in mangrove, *Bruguiera sexangula* found a specific protein allene oxide cyclase (AOC) responsible for enhanced salt tolerance. They designated this protein as mangrin. Further more, expression of mangrin in *Saccharonmyces cerevisae* and tobacco cell lines also

enhanced salt tolerance in these species. Higher contents of soluble proteins have been found in salt tolerant than in salt sensitive cultivars of barley (Hurkman et al., 1991), Sunflower (Ashraf et al., 1995), Finger millet (Uma et al., 1995) and rice (Pareek et al. 1997). In contrast, in lentil, Ashraf and Waheed (1993) reported that leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance. Ashraf and Fatima (1995) found that salt tolerant and salt sensitive accessions of Safflower did not differ significantly in leaf soluble proteins. Similarly, comparison of salt tolerant wild populations with cultivated populations of *Melilotus indica and Eruca sativa* showed that the salt tolerant populations did not differ from salt sensitive populations in soluble proteins content of their leaves at varying salt levels. Pareek et al., (1997) suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering techniques. Many investigators have reported that the proteins produced under salt stress are not always associated with salt tolerance.

Higher plants have been reported to accumulate amino acids under salinity stress (Mansour, 2000 and Ashraf, 1994). The important amino acids include alanine, arginine, glycine, serine, leucine, and valine, together with the amino acid, proline and the non-protein amino acids, citrulline and ornithine. Amides such as glutamine and asparagine have also been reported to accumulate in plants subject to salt stress (Mansour, 2000 and Dubey, 1997). Proline that occurs widely in higher plants accumulates in large amount in salt stressed plants (Ali et al. 1999). Proline accumulation occurs in response to water deficit as well as to salt. Thus, synthesis of proline is a non specific response to low growth medium water potential (Ashraf, 1994). Proline that regulates the accumulation of usable N, is osmotically very active (Ashraf, 1994), contributes to membrane stability (Rudolph et al., 1986; Lone et al., 1987; Hanson, 1994.) and mitigates the effect of NaCl on cell membrane disruption Mansour, (1998). The role of proline in osmoregulation and salt tolerance in general has been questioned. Lutts et al., (1996) observed that proline did not take part in osmotic adjustments in salt-stressed rice and its accumulation seemed to be a symptom of injury rather than indicator for salt tolerance. Similarly Ashraf (1989) reported negative relationship between proline accumulation and salt tolerance in *Vigna mungo*.

Amides accumulate in salt stressed plants in less quantity as compared to other nitrogen containing compounds (Mansour, 2000) and concentration of asparagine frequently increases in response to stress (Fougere et al., 1991, Rabe, 1990). However, the role of amides in salt tolerance mechanism needs to be further elucidated.

The main quaternary ammonium compounds (QACs) that function as effective compatible osmolytes in plants subject to salt stress are glycinebetaine, B-alaninebetaine, prolinebetaine, choline O-sulfate, hydroxyprolinebetaines and pipecolatebetaine (Mansor, 2000, Wyn Jones, 1981, Rhodes 1993). In several plant species, a positive correlation between leaf osmotic potential and glycinebetaine, B–alaninebetaine and prolinebetaine has been observed Rhodes, (1993) and Grieve, (1984). Glycinebetaine (GB) accumulates in response to stress in many crops and is mainly localized in chloroplasts and plays a vital role in chloroplasts adjustment and protection of thylakoid membranes, thereby maintaining photosynthetic efficiency (Robinson, 1986 and Genard, 1991). Many cereals accumulate GB when exposed to stress environments although some plants like rice do not. Accumulation of glycinebetaine under saline conditions was reported to be high in some salt–tolerant grasses but not in salt sensitive grasses (Wyn Jones et al., 1981, Grieve et al., 1984, Hanson, 1985, Rhodes, 1987). Varshney et al., (1988) have shown that accumulation of choline and betaine in response to salt stress was more pronounced in salt sensitive than in slat tolerant lines of *T. alexandrinum.* However, Wyn Jones et al., (1984) did not find any relationship between GB accumulation and salt tolerance of the species of the genera *Triticum, Agropyron* and *Elymus*. In view of these reports it is evident that the occurrence of glycibetaine accumulation is widespread and occurs in large quantity in some plant species whereas its occurrence is doubtful in other species. Thus, to relate the accumulation of GB with plant tolerance requires further investigation.

Among the compatible solutes involved in osmoregulation and mechanisms for increased salt tolerance in plants polyols play on important role (Jefferies, 1980, Gorham et al., 1981 and Bohnert et al., 1999). Polyols are polyhydric alcohols. They exist in both cyclic and acyclic forms and are widely distributed in the plant kingdom. (Sun et al., 1999 and Clark et al., 2003). The most common polyols in plants include acyclic forms, mannitol, glycerol, sorbitol, and cyclic forms, ononitol and pinitol. The polyols are thought to accumulate in the cytoplasm of some halophytes to overcome the osmotic disturbances caused by high concentrations of inorganic ions accumulated in the vacuoles (Gorham et al.. 1981 and Nelson, 1999). Polyols are key to osmoregulation and also function as oxygen scavengers. Mannitol has been reported to act as scavenger of reactive oxygen species *in vitro* (Elstner 1987, Halliwell et al., 1988). It has been shown that mannitol increases the ability of plants to tolerate high salinity. For example in tobacco, which normally does not synthesize or accumulate mannitol, the bacterial mannitol-I- phosphate dehydrogenase (mtlD) gene has been incorporated (Tarczynski et al., 1992). This

transgenic tobacco plant accumulated high concentrations of mannitol in leaves, roots and exhibited a high degree of salt tolerance. Thus, it is evident that mannitol accumulation in plants has a positive association with salinity tolerance.

G.4. Enzyme activity in plant under stress condition:

The use of isozyme as markers has also been an approach of considerable value. Doebly, (1990) advocated that it could aid greatly to the understanding of crop evolution. Apart from being used as markers for population and evolutionary studies isozyme can also be used as putative markers for plant adaptation to extreme environmental conditions like salinity. Variation in salt-tolerance in six natural populations of *Stylosanthes humilis* from three ecogeographic regions of Pernambuco state, northeast Brazil was evaluated on the basis of isozyme study. An electrophoretic analysis of esterase and peroxidase isozyme established a correlation between salt-tolerance and allelic frequencies. The analysis of salt tolerant and salt sensitive families of populations from semi-arid tropical climate suggested an association of alleles of a peroxidase locus with salt-tolerance during germination (Lovato and Martins, 1997). Similarly, variation in the isozymes composition of peroxidase, catalases, esterases, amylases, and glutamate and malate dehydrogenase was studied in the callus and seedling phase of the rice (*Oryza sativa*) varieties J104, J112, Pokkali, Amistad 82, IR 36 and soma clones of Amistad 82, with different salt treatments. PAGE analysis revealed quantitative and qualitative variations in amylase composition in the calluses and esterase composition in the root tissue of seedlings under salt-stress. There was no appreciable isozymic variation in the leaf tissue of the tested varieties and soma clones (Iglesias and Gonzalez, 1995).

Peroxidase isozymic pattern of root cultures of Shangnong new lines of rye grass (*L. multiflorum)* in 0 and 0.3% salt solutions demonstrated that the number 7 bands are characteristics of population in the 0.3% salt solutions and that the number 7 band of the new lines are darker brown than those of the controls (He et al., 1992).

The properties of polyphenol oxidase (PPO), IAA oxidase and catalase activities studied *in situ* and *in vitro* from seedlings of 2 salt sensitive and 2 salt tolerant cultivars raised under increasing levels of salinity indicated that salinity *in situ* caused 20-100% increase in IAA oxides activity in roots, with a greater increase in sensitive cultivars than tolerant ones. Salt tolerant seedlings maintained a lower level of IAA oxidase activity than the sensitive ones under control as well as in salt treatments. NaCl *in vitro* caused about 22-85% inhibition in enzyme activity with lesser inhibition in sensitive cultivars than in tolerant ones. Catalase activity was higher in seedlings of salt-tolerant cultivars than in

sensitive cultivars. Root maintained a higher level of catalase activity than shoots. It was proposed that the direction of change in the activity of enzymes between roots and shoots change within the cultivars (Mittal and Dubey, 1995).

The active oxygen species such as superoxide (O_2^-), hydrogen peroxide (H_2O_2), hydroxyl radical (OH), peroxy radicals, alkoxy radicals, singlet oxygen etc. are generated in plants in response to different environmental stresses such as salinity, drought, water logging, temperature extreme, high light intensity or mineral nutrient deficiency. (Spychalla et al., 1990 and Mittova et al., 2002). Generation of ROS is one of the earliest responses of plants cells under various abiotic and biotic stresses. Plants with high concentrations of antioxidants show considerable resistance to oxidative damage caused by activated oxygen species.

Dionisiosese and Tobita, (1998) reported decline in SOD activity and an increase in peroxidase activity in the salt sensitive rice varieties in response to salt-stress, whereas the salt tolerant varieties Pokkali and Bankat showed only slight increase in SOD but a slight decrease in peroxidase activity.

Higher concentrations of catalase and $\propto$-tocopherol were found in salt tolerant lines of cotton as compared to the salt-sensitive lines, salt stress also caused a considerable increase in the activities of peroxidase and glutathione reductase in the salt tolerant cultivars, whereas the activities of these enzymes remained unchanged or decreased in salt sensitive cultivars. (Gooset et al., 1994). They advocated that high levels of the antioxidants are associated with salt tolerance in cotton. Increased salinity led to significant increases in SOD, POD and GR activities in Pora cultivars of cotton, but they remained unchanged in Guazuncho (Meloni et al., 2002). They concluded that the difference of SOD, POD and GR activities in the two cotton cultivars could be ascribed to the difference in mechanisms underlying oxidative stress injury and subsequent tolerance to salinity. It was inferred that Pora cultivars, which exhibited higher salt tolerance, had also higher antioxidant enzyme activity than Guazuncho. A relationship between lipid peroxidation, the antioxidant defence system and salt stress in salt- sensitive cultivated tomato (*Lycopersicon esculentum*) and its salt-tolerant wild relative (*L. pennellii*) was established. Superoxide (SOD) activities were significantly higher in the leaves of *L. pennellii* than those of *L. esculentum* after 12 and 84 days. POX activity showed a gradual increase in both cultivars under 70mM NaCl. POX activity in *L. pennellii* significantly increased after 6 and 84 days whereas showed no remarkable changes in leaves of *L.*

esculentum under 140mM NaCl. A higher salinity tolerance of *L. pennellii* was also correlated with a lower lipid peroxidation, which might be due to a higher content of antioxidant enzymes. (Koca et al. 2006).

Cavalcanti et al. (2004) advocated that the ability of cowpea (*Vigna unguiculata)* plants to survive under high levels of salinity is not caused by an operating antioxidant system involving SOD, POX and catalase activities in mature leaves.

In view of the considerable variations in the protective mechanism against activated oxygen species in different plant species, further work is required to establish the general validity of this phenomenon in salinity tolerance.

H. Molecular Markers in Salinity Tolerance

Plants respond to salt stress at three different levels i.e., cellular, tissue and whole plant level. Cell based mechanisms of ion homeostasis and the synthesis of different osmoprotectants are regarded as essential determinants for salt tolerance but the adaptive mechanisms to tolerate salt stress may vary from species to species. Therefore, separate study of each level of response at cellular, tissue and at whole plant level may be the best way to correctly place the pieces together to understand the whole picture of salt-tolerance. As salt tolerance is regulated throughout the plant development and is more a tissue specific phenomenon, plant's response at one stage of development is not necessarily the same at other stages (Johnson et al., 1992; Lauchli and Epstein, 1990). Therefore, the mechanisms of salt tolerance at specific stages of plant development must be studied. Genetic studies have demonstrated that the ability of plants to tolerate salt stress is a quantitative trait involving the action of many genes. This situation has been complicated by the fact that the main character selected in crop plants has been productivity, which is also a complex trait. Therefore, the integration of genes required to increase salt tolerance in a specific genotype is difficult without appropriating other important multigeneic traits like flowering, fruit quality and dry matter production (Flowers et al., 2000). Therefore, an important objective should be to determine the limiting factors and key processes that produce salt tolerance, i.e., salt tolerant determinants. The principal approach employed to ascertain salt tolerance determinants in plants was to identify the metabolic processes critical to tolerate NaCl. Maintenance of ion homeostasis is the foremost requirement for plants to thrive under salt stress conditions. Plant cells respond to salt stress by increasing Na^+ efflux at the plasma membrane and Na^+ accumulation in the vacuoles. Therefore, proteins and ultimately,

genes involved in these processes can be considered as salt tolerant determinants. Another metabolic response to salt stress is the synthesis of compatible osmolytes. These organic compounds have been investigated to mediate the osmotic adjustment required in protecting sub-cellular structures and oxidative damage by their free radical scavenging capacity (Smirnoff, 1993; Hare et al., 1998 and Hong et al., 1992). Therefore, the genes regulating the accumulation of these organic compounds can be considered as salt tolerant determinants.

Recent advances in molecular biology have broadened the possibilities for gene manipulation at the cellular and higher units of organization. The developments of new and improved technologies now make gene identification, isolation and transformation realities. The major problem that prohibits the use of these technologies to develop salt tolerant crops is that salt tolerance is a complex, multigeneic trait and is a composite response of the integrated biological system. However, molecular markers are good indicators of response to biotic and abiotic stresses and are being recently used for fast identification of trails for salt tolerance.

The selection based on molecular markers is known as marker assisted selection (MAS). The MAS helps in overcoming the difficulties associated with low heritability, recessive ness and difficult screening assays during the gene transfer and selection. Since most of the morphological and physiological trails associated with a biotic stress resistance as root development or osmotic adjustment are difficult to manipulate using conventional screening methods. The use of recombinant DNA technology can be successfully applied to crop improvement for stress tolerance. Genetic studies of complex trails such as salt – tolerance, have become easier with the development of informative molecular markers such as RFLP; RAPD; Simple Sequence Repeats (SSR); AFLP etc have provided a new level of precision in genotyping and offer great potential in studies.

Zhong et al., (1997) used DNA amplification finger printing to screen two salt sensitive cultivars (Hark and Jackson) and two salt-tolerant cultivars (Morgan and Wengfang 7) with the aim of identifying polymorphic markers to indicate salt-tolerance. The polymorphic markers 8.6f/350 bp, 8-27/24 bp and 8-15/215bp appeared only in the salt tolerant cultivars. A modified RAPD procedure revealed a higher level of genetic diversity in wild soybean populations. It was proposed that the high level of genetic diversity and developmental flexibility of wild soybean is responsible for its successful adaptation to changing salinity (Wang et al., 1997). Similarly genetic screening of 4 rice varieties using RAPD markers for identification of salt sensitive/resistance was carried by

Polymerase chain reaction. Genetically distinct fingerprints were constructed on the basis of co-segregation occurring between specific banding patterns and salt tolerance. The fingerprints produced in these reactions showed large number of putative markers (Erikson et al., 1995).

A co-dominant marker, which was able to distinguish heterozygous and homozygous individuals, was successfully identified using RAPD markers linked to salt tolerance in soybean (Guo Pei et al., 1998). No recombinants were found in 43 salt sensitive genotypes analyzed using this marker, suggesting that the marker is tightly linked with the salt tolerance genes or within it.

As it is a known fact that a biotic stress tolerance are quantitatively inherited traits, controlled by several genetic loci, (QTLS). RFLP markers have proved to be useful in the mapping of quantitative traits and will be useful in determining the number of chromosomal segments involved in the resistance phenotype. The introgression of alien germplasm can be monitored using RFLP markers both for the segment(s) of interest and for the size of the population needed to be carried through a selection scheme. The inheritance of salt tolerance in rice was studied using a tolerant rice mutant (M-20) obtained through selection *in vitro.* Its tolerance was stably inherited over 8 generations and most traits between M-20 and its sensitive original 77-170 (*Oryza sativa*) were similar under saline conditions the segregation among F2 individuals was obvious. The ratio of salt sensitive moderately tolerant plants was in the 1:2:1 ratio. It is suggested that the improvement of salt tolerance in this method was induced by the mutation of a major tolerant gene, which showed incomplete dominance. By using 130 RFLP probes distributed throughout the rice genome, the gene was tagged by a single copy of DNA probe, RG 4, which was located on the chromosome 7. The genetic distance between the salt tolerant gene and RG 4 was 7-01≠ 2.9 cm. Based on the split method, a method which could be used to evaluate the damage of salt-stress in rice is proposed (Zhang et al., 1995).

RFLP markers can be employed to detect genetic loci (often referred to as quantitative trail loci (QTL's) underlying quantitative traits in a conceptually simple manner. The ability to detect a QTL with an RFLP marker is a function of the magnitude of the QTLs effect on the character, the size of the population being studied and the recombination frequencies between the marker and the QTL. Recombinant inbred (Rl) lines developed by single seed descent procedure from the cross Tesanai 2 x CB was used to map QTLs

controlling salt tolerance in rice. The 142 lines were genotyped by 60 RFLP markers and evaluated for seedling survival days (SD) in culture solution with EC of 12 ds/m. Transgressive segregations were observed for salt tolerance in the RI populations. A linkage map consisting of 52 marker loci from 11 linkage groups was constructed. Only marker locus RG 132 on chromosome 5, which associated significantly with salt tolerance was detected and explained 11.6% of the observed phenotypic variance for SD. Quantitative trait loci (QTLs) controlling salt tolerance at germination and the seedling stage in barley were identified by interval mapping analysis using marker information from 2 doubled haploid (DH). Interval mapping analysis revealed that the QTLs for salt tolerance at germination and at the seedling stage were controlled by different loci (Mano et al., 1997).

Interest has now centered on SSRs as a genetic marker system (Tautz and Renz, 1984). SSRs are PCR based markers, which have the advantage of being single locus markers, co-dominant, multi-allelic and widely dispersed over the genome. In barley SSRs have been developed and there are now 500 mapped populations (Waugh et al., 1997).

QTL mapping has evolved to become a standard procedure in dissecting the genetic controls of a variety of traits. The next step should be the identification of the genes, alleles and physiological processes that are important for stress tolerance in agriculture. The genomic regions identified by QTL analysis can be surveyed for coincident candidate genes of known function; unlinked functional genes can be eliminated. Comparing genotypes, which vary for critical genomic regions, can perform more detailed studies and useful traits (morphological, developmental, physiological and biochemical) can be identified which contribute to yield under stress conditions. If QTLs for stress responses are coincident with commercially important traits then there may arise problem in changing these in favour of more potent stress response alleles. Therefore, a more effective strategy may be to target stress QTLs unlinked to commercially important QTLs.

Monforte et al., (1996) studied the usefulness of marker- assisted selection (MAS) to develop salt tolerant breeding lines from a F_2 derived from *L. esculentum* and *L. pimpinellifolium.* Interval mapping methodology of quantitative trail loci (QTL) analysis was used to locate more precisely salt tolerance QTLs. A new QTL for total fruit weight under salinity (TW) near TG 24 was detected. Most of the detected QTLs (3 for TW, 5 for fruit number (FN) and 4 for fruit weight (FW) had low R^2 values, except the FW QTL in the TG 180- TG 48 interval. Dominant and over dominant effects were detected at the

QTLs for TW, whereas gene effects at the QTLs for FN and FW ranged from additive to partial dominance. Phenotypic selection of F2 families and marker – assisted selection of F3 families were carried out. Comparison of the yield of these families under control versus saline conditions showed that fruit weight is a key trait to success in tomato salt tolerance improvement using wild *Lycopersicon* germplasm.

As for the QTL identified for fruit yield, QTL associated with germination depend upon the conditions under which germination is assessed (Foolad et al., 1999). A similar situation exists for citrus, where about half of the potential QTL identified depended on the presence on absence of salinity (Tozlu et al., 1999) and in rice (Gong et al., 1999, 2001) where less than 10% of the QTL were detected both in the presence and absence of salt. Thus, the major determinants of yield vary with the environmental conditions and quantitative traits typically exhibit a large environment x genotype interaction. The use of tomato has also been important in establishing that QTL associated with tolerance vary with the stage of plant development. Thus, the use of QTLs has improved the efficiency of selection, in particular for those trails that are controlled by several genes and are highly influenced by environmental factors (Flowers, 2004).

I. Methods for improving salt –tolerance

Salt stress is certainly one of the most serious environmental factors limiting the productivity of crop plants (Ashraf, 1994). Despite the advances in the increase of plant productivity and resistance to number of pests and diseases, improvement in salt tolerance of crop plants remains elusive. It is a known fact that earth is a salty planet, with most of its water containing about 30 g of sodium chloride per litre. This salt solution has affected and continues to affect, the land on which crops are grown. Consequently salinity is a threat to food supply. Growth of human population by 50% from 6.1 billion in mid-2001 to 9.3 billion by 2050 (http://www.unfpa.org/swp/2001/) means that crop production must increase if food security is to be ensured. Given the amount by which food production will have to be increased, it seems reasonable to predict that changing the salt-tolerance of crops will be an important aspect of plant breeding in the future, if global food production is to be maintained.

Efforts to improve crop performance under environmental stresses have not been that fruitful because the fundamental mechanism of stress tolerance in plants remains to be completely understood. Twenty-five years ago Emanuel Epstein (1980), described the technical and biological constraints to solving the problem of salinity. Although there has

been some success with the technical solutions to the problem, the biological solutions have been more difficult to develop because a pre-requisite for the development of salt-tolerant crops is the identification of key determinants of stress tolerance. Flowers and Yeo (1995) suggested five possible ways to develop salt tolerant crops:

1. Develop halophytes as alternative crops. They advocated that it may be more successful and cheaper, to domesticate a wild salt –tolerant species than modify an existing crop species to grow productively in a saline environment. Domestication has a well-documented track record of success and the rate of progress with triticale is well known. O′ Leary (1994).
2. Use interspecific hybridization to raise the tolerance of current crops.
3. Use of variation already present in existing crops.
4. Generate variation within existing crops by using recurrent selection, mutagenesis or tissue culture.
5. Breed for yield rather than tolerance.

These all remain possible solutions to the problem. Although conventional form of breeding have not, in general, delivered salt-tolerant genotypes. Bohnert and Jensen (1996) claimed that an important approach has been missed by Flowers and Yeo. They wrote, tolerance breeding must be accompanied by transformation and that successful releases of tolerant crops will require large scale "Metabolic engineering" which must include the transfer of many genes.

Two basic genetic approaches are currently being utilized to improve stress tolerance:

1. Exploitation of natural genetic variations, either through direct selection in stressful environment or through the mapping of quantitative trait loci (QTLs) and subsequent marker assisted selection.
2. Generation of transgenic plants to introduce novel genes or to alter expression levels of the existing genes to affect the degree of salt stress tolerance.

I.1. Marker-assisted breeding:

The selection of superior salt-tolerant genotypes under field conditions is handicapped by the significant influence of environmental factors on plants (Richards, 1996). Salt tolerance in plants appears to be a developmentally regulated process and the tolerance of the plants at one stage of development is not always correlated with tolerance at other stages (Foolad 2004, Flowers 2004, Greenway and Munns 1980, Tal and Shannon, 1983).

The development of molecular biology techniques has enabled the development of DNA markers that can be used to identify QTLs. The use of QTLs has improved the efficiency of selection, in particular for those traits that are multigeneic and are highly influenced by environmental factors (Flowers, 2004). QTLs and marker assisted selection provide several advantages over direct phenotypic screening, particularly because the PCR-based methodologies used to detect the markers reduce the time needed to screen individuals and reduce the impact of environmental factors on the traits under study. The multigeneic nature of salt tolerance has already been established and quantitative trait loci associated with aspects of germination, ion transport and yield identified. Salt tolerance and its sub-traits are determined by multiple QTLs and that both additive and dominance effects are important in the inheritance of many traits associated with salt tolerance. (Foolad 2004, Flowers 2004, Gregorio, et. al. 2002). The development of high-density DNA maps that incorporate micro satellite markers, RFLP and AFLP and advances in marker assisted selection techniques will facilitate pyramiding traits of interest to attain substantial improvement in crop tolerance.

I.2. Improving salt tolerance - transgenic approach:

Salt tolerance of plants depends primarily on characteristics that can be broadly grouped in three categories.

1. Physical uptake on exclusion of salt followed by transport and their compartmentation.
2. Morphological features and biomass distribution of plant shoots and roots.
3. Physiological and metabolic events that counteract the presence of salt at the cellular/ tissue level.

These characteristics can be targeted for manipulation in engineering of salt/drought tolerance. Plant morphological adaptations and salt transport in the xylem depends on a complex pattern of developmental regulation and have so far received little attention with molecular techniques. Guard cell responses to environmental stimuli have been well documented by (Kearns and Assman, 1993), but these responses cannot be manipulated in a heritable fashion. General inhibition of shoot growth with continued root growth has been considered as a morphological adaptation to salt stress and water deficit (Creelman et al., 1990). Enhanced root development could be beneficial in salt/drought tolerance as indicated from studies on adaptation but molecular techniques for effectively manipulating root mass have not been developed (Aeschbacher et al., 1994). Ion uptake and transfer across membrane has been investigated as integral metabolic changes in salt

stress and adaptation (Niu et al., 1995). Membrane components of ion pump could thus be encoded by a group of genes which, when activated, would counteract acute salt stress.

Physiologic or metabolic adaptations to salt stress at the cellular level are the main responses amenable to molecular analysis and have led to the identification of a large number of genes induced by salt (Ingram and Bartels 1996; Bray 1997; Shinozaki and Yamaguchi 1996). These genes can be classified in groups related to their physiologic or metabolic functions

Some of the functional groups of genes/proteins activated in salt stress with potential for providing tolerance are: Carbon metabolism and energy production/photosynthesis, Cell wall/membrane structural components, osmoprotectants and molecular chaperons, Water channel proteins, Ion transport, Oxidative stress defenses, Detoxifying enzymes, Proteinases, Proteins involved in signaling and Transcription factors. Most of the genes in the functional group have been identified as salt inducible under stress conditions. Other genes have been detected by a salt hypersensitivity assay in *Arabidopsis*. Primarily these genes encode enzymes involved in osmoprotectant synthesis, molecular chaperons and detoxifying enzymes involved in oxidative stress responses. Increased osmoprotectant synthesis has been manipulated in plants by over expression of enzymes leading to increased mannitol synthesis in tobacco (Tarczynski et al. 1993) and *Arabidopsis* (Thomas et al., 1995), amino acid proline in tobacco (Kishor et al., 1995) etc. The transgenic plants accumulating proline demonstrated that degradative as well as synthetic pathways might need to be manipulated if constitutively higher than normal proline levels were to be attained. In Maize osmoprotectant glycinebetaine accumulation has been shown to correlate with Bet1 gene copy number and improved salt tolerance (Saneoka et al., 1995) similarly it was reported by Petrusa and Winicov (1997) that salt tolerant alfalfa plants rapidly double their proline concentrations in the roots, while salt–sensitive plants had a delayed response. Late embryogenesis abundant (LEA) proteins are thought to play a role in desiccation tolerance in seed development and in response to dehydration, salinity and cold stress (Close, 1997). Rice plants transformed with the barley LEA gene, HVAI, have shown increased tolerance to water deficit and salt–stress (Xu et al., 1996). Improved salt and freezing tolerance has been observed in yeast transformed with a tomato LEA-class gene (Imai et al., 1996). These proteins are thought to preserve the integrity of cell. Since cell wall is a fundamental structure in response to environmental stresses, as cell wall properties like water permeability and elasticity are involved in the

maintenance of cell growth during salt stresses (Iraki et al., 1989), alteration in the cell wall might be crucial to stress tolerance.

Salinity generates an increase in reactive oxygen species (ROS) that induce deleterious effects on cell metabolism. Various group of workers have developed plants that over–express several oxidative stress related genes, with varied results. The potential role of super oxide dismutase (SOD) in the protection against salt stress has been investigated using transgenic rice plants (Tanaka et al., 1999). At high salinity, the transgenic plant had 1.5-fold higher ascorbate peroxidase activity than the control plants. Total SOD activity was maintained at a high level and ascorbate peroxidase increased under salt-stress. It was found that the PS II activity and the electron transport in the chloroplast were higher in the transgenic plants compared to the wild plants under stress. This suggests that an increase in the levels of ascorbate peroxidase and chloroplastic SOD are important factors for salt resistance in rice. Similarly transgenic tobacco seedlings over expressing glutathione S-transferase (SST) and glutathione peroxidase (GPX) have been generated. Over expression of glutathione reductase, in transgenic plants leads to elevated levels of GSH, increasing tolerance to salt and oxidative stress in leaves (Foyer et. al., 1991, 1994).

An alternative approach to generate plant salt tolerance is the introduction of genes that regulate ion transport system such as HAL 1 (Gisbert et. al., 2000; Yang et al., 2001) and HAL 3 (Albert et al., 2000) from *Saccharomyces cerevisiae.* Over expression of HAL1 in tomato improved salt tolerance by maintaining a high internal K^+ concentration and decreasing intracellular Na^+ during salt stress (Gisbert et. al., 2000). Though Na^+ is required in some plants, particularly halophytes, a high concentration of NaCl is toxic and affects plant growth (Glenn, et. al., 1999). The alteration of ion ratios in plants is due to the influx of Na^+ through pathways that function in the acquisition of K^+ (Blumwald, 1987). The sensitivity of cytosolic enzymes to salt is similar to both glycophytes and halophytes, indicating that the maintenance of a high cytosolic K^+/Na^+ concentration ratio is a key requirement for plant growth in soils with high concentration of salt. Processes that plants could use to maintain a high K^+/Na^+ ratio in the cytosol include:

1. Extrusion of Na^+ ions out of the cell
2. Vacuolar compartmentation of Na^+ ions.

Plant cells are structurally well suited for the sequestration of ions because of the presence of large membrane bound vacuoles. Over expression of a vacuolar Na+/H+ antiport from *Arabidopsis thaliana* (AtNHXI) plants promoted sustained growth and

development in soil having 200 mM NaCl (Apse et. al., 1999). Tomato plants over expressing the same gene were able to grow, flower and produce fruit in the presence of 200 mm NaCl (Zhang and Blumwald, 2001). Though the leaves accumulated high concentration of sodium, the tomato fruits displayed low amounts of sodium. Similar results were obtained with transgenic *Brassica rapus* (Canola) over expressing AtNHX1 (Zhang et. al., 2001). Sodium accumulated in the leaves of transgenic plants grown in the presence of 200 mM NaCl formed up to 6% of the dry leaf weight, but the seed yields and oil quality were not affected, demonstrating the potential use of this technology for agricultural use in salt affected soils.

Another attractive target category for manipulation and coordinate gene regulation is the small group of transcription factors that have been identified to bind to promoter regulatory elements in genes regulated by salt/drought stress (Shinozaki and Yamaguchi, 1997). The transcription factor DREB IA (Dehydration Response Element Binding) specifically interacts with the DRE (Dehydration Response Element) box promotion sequences and induces expression of stress tolerance genes with DRE elements in their promoters. The over expression of DREB IA cDNA in *Arabidopsis* plants activated the expression of many stress tolerance genes under normal growing conditions (Liu et. al., 1998). Transgenic plants with DREB IA ectopically expressed under the control of the CAMV 35S promoter showed morphological abnormalities under unstressed conditions. In contrast, plants expressing DREB IA under the control of the salt inducible rd 29A promoter looked healthy and exhibited high tolerance to salt stress (Kasuga et. al., 1999). This indicates that stress inducible promoters may be more desirable in order to generate plants that are tolerant to stress.

Transgenic approaches for increasing plant salt tolerance are promising with the recognition that the enhanced expression of a number of functionally related genes may be required for optimal improvement in salt tolerance; molecular engineering has been expanded to include proposals for multiple genes transfer to enhance salt tolerance (Bohnert and Jensen, 1996). An equally promising approach to manipulating many genes may emerge as we learn more about the specific signaling pathways that turn on transcription of related genes that counteracts salt stress at the cellular level and further in a tissue targeted levels.

I.3. Use of *in vitro* selection for salt –tolerance

Much progress has been made in the last decade in attempt to use cell and tissue culture for the improvement of salinity resistance. Many of the technical problems are being

solved. Several laboratories around the world are actively involved in this area and encouraging results can be expected. The development of a practical contribution by this method in the form of a viable commercial, resistant cultivar, or at least a valuable resistant parental line, is not far away.

Selecting cultured cells for survival at high NaCl potentially offers a fast means for generating, evaluation and selecting genotypes with superior salt tolerance. Hasegawa et al., (1995) advocated improvement of salt tolerance in plants by application of tissue culture to obtain salt tolerant plants and for identification and characterization of cellular determinants of salt-tolerance. Dunkan et al. (1995) have in a study on Sorghum advocated that *in vitro* cell selection and somaclonal variation offer an alternative to traditional breeding methodology for generating improved breeding lines for hybrid development. Al-Khatib et al. (1994) screened 35 cultivars of Lucerne (*Medicago sativa*) to increasing NaCl concentration. Shoot length was found to significantly decrease with increasing NaCl concentration. They advocated selection between and within cultivars might lead to increased salt tolerance in this species. Rapid *in vitro* screening of 14 cultivars of *Triticum aestivum* using excised mature embryos for salt tolerance was reported by Diaz et al. (1995). The germinated seedlings after 8 days were evaluated for height, root length and root number. Cultivars were classified as tolerant (0-35% inhibition compared with control values), moderately tolerant (36-68% inhibition) or sensitive (69-100% inhibition). Prolonging the selection process *in vitro* in rice has been reported to improve the likelihood of regenerating plants with improved salt tolerance (Winicov, 1996). Successive subcultures of explants initiated from Lucerne leaflet (*Medicago media* cv. Rambler) into media of progressively higher salinity resulted in regenerants, which were more salt tolerant than the original, unselected Rambler plants (Chaudhary et.al., 1996). Sub culturing of the variant lines derived *in vitro* from unorganized tissues of potato (*Solanum tuberosum* + *S. chacoense*) somatic hybrids from callus tissues preincubated on medium containing 1.5% NaCl significantly surpassed the hybrid in shoot development. Numerous tests of the selected plant lines over several years confirmed their salt tolerance. It was advocated that the higher tolerance of the selected variant lines was due to cell selection for tolerance to NaCl. (Burgutin et al., 1996). Different explants of two cotton varieties responded similarly to NaCl treatment. Low NaCl concentration had little effect while high concentration inhibited the initiation and growth of calluses. The sensitivity of the explants to NaCl was in the order radicle>hypocotyls> cotyledon (Wang et.al., 1991). Similarly, plant regeneration of salt

adapted callus of Indica rice (var Basmati 370) under varying NaCl concentrations was studied by Basu et al. (1997). Calli isolated from immature embryos of four wheat cultivars under varying NaCl concentrations indicated that the relative growth rate of callus decreased as the concentration of NaCl increased in both selected and unselected callus lines. The selected callus line gave a higher growth with presence of NaCl in the medium and was highly significant as compared with unselected callus lines. The dry weight of both kinds of callus lines of all wheat cultivars increased markedly with increasing NaCl concentration in most cases (Barakat and Latif 1996). Similar results were obtained in maize by Ivanova and Petrova (1995). Somatic embryos, developed from hypocotyls segment of light grown seedlings of *Brassica juncea* cv. RLM 198, were subjected to selection at varying concentrations of NaCl. Plants were developed from proliferated somatic embryos selected on NaCl-containing medium (Kirti et al., 1990). It was concluded that selected tolerant lines showed better root growth, shoot growth, and fresh weight accumulation on salt-containing medium when compared to the control and salt tolerance was transmitted to the next generation in seed progeny of tolerant plants grown in the absence of salt.

Effect of salinity was investigated on four Moroccan wheat cultivars Karim, Sebou (durum wheat), Sais and Merchouch (soft wheat). Salt stress was applied to five-week-old callus, which were cultivated in media deprived of salt. Regeneration was studied in media treated or untreated with salt. (Oudija et al., 2002). Results showed a decrease in embryogenesis ability at high salt concentrations (10 and 15 g/l) in all cultivars. The higher the salt stress, lower were relative growth and somatic embryogenesis. At 15 g/l most cells showed necrosis and degenerated. Soft wheat tolerated more salt than durum wheat. The best regeneration results were obtained in media containing 0 or 2.5 g/l of salt. Browning started to show on the seedlings at 5 g/l and was complete at 15 g/liter.

One month old callus of wheat cultivars Lu-265 and Potohar in MS liquid medium were subjected to different salt concentrations to assess the effects of salinity stress on growth, ion accumulation, accumulation of organic solutes and relative water content (RWC). The growth rate of callus decreased with increasing salt concentrations, callus Na^+ and Cl^- increased, whereas K^+ decreased with increasing salt concentrations. Callus K^+: Na^+ ratio decreased with increasing salt concentrations. The total soluble proteins, carbohydrates and amino acids in the callus of both cultivars increased with increasing salt concentration. The relative water content (RWC) of both callus decreased with increasing

salt concentrations with Potahar recording higher RWC reduction than Lu – 265 (Farrukh Javed 2002).

To study the relationships between different traits and salt tolerance in bread wheat through tissue culture and germination test, two experiments were conducted. In the first experiment, 5 salt-tolerant cultivars and 3 salt sensitive cultivars were employed. The NaCl rates were taken as 0, 0.38, 0.77 and 1.15%. The results showed that salt-stress had no effects on either callus fresh weight or callus number. Callus weight did not change with salt stress, but increasing the salt stress decreased the callus sizes and increased Na^+ content of the callus. Genotypes x salt effects were significant for K^+ and K^+/Na^+ ratio. The study showed that variation in K^+ in callus was related to tolerance or sensitivity of cultivars and hence indirect selection of salt-tolerant lines may be done through this approach. The germination experiment conducted revealed that traits such as germination percentage, germination rate, coleoptile's length and root length had higher growth rates in the tolerant cultivars (Ghannadha et al., 2005).

In vitro culture (anther culture) response of 30 F1 hybrids from crosses between salt tolerant and salt susceptible rice varieties was studied on N_6, B_5 and MS media with varying concentrations of auxins and cytokinins. In most crosses 2, 4-D (Mostly at 2 mg/liter) with a low level of kinetin was the best combination for inducing androgenic calluses. 13 hybrids did not produce callus reaffirming that androgenic callus response is largely genotype dependent. Callus producing ability was in the range of 0.83 – 0.84%. Average number of callus per responding anther was in the range of 1.60 – 4.5. Callus initiation required 19-36 days. Plant regeneration percentage ranged from 28.6 to 72.6 in MS media with different concentrations of kinetin and BAP (benzyl adenine). Albinos were abundant among the regenerants. With such low callus formation and plant regeneration abilities producing 150 double haploids (DH) lines (Minimum for effective field screening) appeared difficult therefore large scale experimentation for producing large number of regenerants is required (Mandal et al., 1997).

The possible use of *in vitro* shoot morphogenesis and shoot apex culture to evaluate salt tolerance in cultivated tomato (*Lycopersicon esculentum* Mill.) has been analyzed, using two cultivars with similar salt tolerance, Pera and Hellfrucht Fruhstamm (HF). The effect of salt on shoot regeneration was studied by culturing leaf explants on media supplemented with 0, 43, 86, 129 and 172 mM NaCl. The presence of NaCl in the regenerating media at 86 mM strongly inhibited shoot regeneration in the cultivar HF, but not in Pera. Root formation was the parameter most affected by salt in both the cultivars.

Furthermore, the salt sensitivity of tomato apices increased with time in culture. The threshold NaCl concentration that significantly affected rooting varied among cultivars and subcultures (Mercado et al., 2000).

Salt tolerance adaptation development in cotton cultivars (*Gossypium hirsutum L.*) through *in vitro* regeneration was undertaken to develop protocol for consistent production of salt tolerant plants through direct organogenesis. 25 mM to 200 mM of NaCl were tested in this study and salt tolerant cotton plants were established through shoot tip, cotyledonary node and leaf node explants. The salt tolerant plants were selected between 100 mM to 175 mM of NaCl treated cultures. The maximum percentage of explants death was observed during rooting compared with multiple shoot induction and shoot elongation. The number of multiple shoot formation was also contrastingly decreased in both the varieties during salt stress (Ganeshan and Jayabalan 2005).

Effect of increasing salt concentrations on the growth of embryogenic calli isolated from immature embryos of four wheat cultivars indicated that stepwise method was most effective in selecting for salt tolerance. It was observed that the relative growth weight of calli was highly significantly influenced by genotype, culture medium and NaCl concentrations (Barakat and Latif, 1985). The best plant regeneration (simultaneous root and shoot formation) rates in mature wheat embryos were observed when calli were induced on 2 mg 2-4 D/L and subculture on 1 mg NAA and 3 g NaCl/L (Kintzios et al., 1997).

Salt tolerance screening of anther derived callus on 7 levels of NaCl (0.0-1.5%) in rice (*Oryza sativa* L.) variety IR-50 suggested that high levels of NaCl (0.9 – 1.5%) reduced callus survival and increased the frequency of albino plants. Low levels of NaCl (0.3-0.6%) resulted in almost equal proportion of green and albino plants. The fresh mass and water contents of callus were drastically reduced at higher salt concentration. The relative high number of green plants regenerated after 2 passages at 0.3 – 0.5 % NaCl suggested that the culture media consisting of MS medium supplemented with 9.3 μM Kinetin, 2.7 μM NAA, 2% sucrose and 3% sorbitol is affective in reducing the frequency of albino plants which is a major problem in screening *in vitro* (Krishnaraj and Sreeranganswamy, 1993). Successful salt tolerant wheat lines were obtained by anther culture under varying levels of NaCl concentration (0.1, 0.3 and 0.5%) by Zao et al. (1995). Gradual increase in salt concentrations produced more and more salt tolerant variants and the tolerance was stably inherited for 5 generations in 25% of variants. Chen et al., (2002) investigated the

salt tolerance in cotyledon-derived callus tolerant to 225 mMol NaCl/L were selected *in vitro* and regenerated into plants. Ten plants regenerated from salt-tolerant callus showed high tolerance when subjected to NaCl stress. Plants maintained their tolerance following transplant to pots and further treated with 150 m Mol NaCl/L. Only one salt tolerant regenerants flowered and fruited and transferred tolerance into their progeny following pollination.

Concern has been raised whether variation in salinity resistance in plants is correspondingly expressed in cell and tissue culture. Several workers have reported that the association may exist. The first stage after the establishment of a cell culture from any suitable source material is the induction and isolation of salt resistant cell lines in the culture. Salinity stress is commonly applied by the addition of NaCl to the culture media. To eliminate variants that have only some transient adaptation to the saline culture, salinity stress has to be high and prolonged enough to kill more than 90-95% of the cells. The surviving cells are repopulated and reselected until certain resistant cell lines are recovered. Cell lines may be reselected under further salinity stress. Salt tolerant cell lines have been isolated in numerous crop plants by different workers. The stability of the salt resistant cell lines has to be established by passing the culture through non-saline media and back to saline media. Resistance must be retained through several such passes.

The next step involves the regeneration of resistant plants from resistant cell lines. Plant regeneration from cell lines is well established in some plants (e.g. Tobacco) but becomes very difficult in some (e.g. most cereals). It is therefore, advisable to first establish varieties with high regeneration capacity before work in cell cultures is initiated.

The passage from cells to callus to plantlets and finally to plants under natural conditions involves elaborate manipulations of culture media and growing conditions. Plant regeneration *en masse* from cell lines has been possible for some species. Regenerated plantlets or plants may or may not carry resistance. Adaptation to salinity, which was retained in cell lines or even in regenerated plants, was often ascribed to be epigenetic changes, which could not be transmitted through sexual reproduction. Chimeral variants were often obtained which were not transmitted to the next generation through sexual reproduction. The transfer of salinity resistance through sexual reproduction and preferably through crosses may be considered as the "acid test" of the whole program of selection *in vitro*.

J. Salinity response to seedling growth

Salinity affects all stages of development and sensitivity varies from one growth stage to another. Some crop species show tolerant behaviour at germination stage but not at later stages of development. Sugar beet, barley and cotton for instance are regarded as the most salt tolerant agricultural crops but each is relatively sensitive during either germination or early seedling growth. Rice may be sensitive during seedling and flowering stages (Pearson, 1966). Pea, grams and beans are not as sensitive during germination as during later stages of development. Piruzyan et al. (1959) observed that corn grown in natural saline soils was most sensitive during emergence and seedling growth but become more tolerant by the flowering stage. They suggested that corn is most sensitive during vegetative growth stage. Salinity tolerance during germination and early seedling growth was evaluated for 24 accessions representing four wild *Phaseolus* species and four accessions of cultivated common bean (*P. vulgaris L.)* at 0, 60, 120 and 180 mM NaCl, by Bayuelo-Jimenez et al., (2002). They observed that salinity stress delayed germination in all accession to varying degrees and exhibited high genetic potential within *Phaseolus* for salinity tolerance during germination. The biomass of radicles plus hypocotyls decreased with increasing salinity but salt stress inhibited hypocotyls growth more than radicle growth. The magnitude of reduction was highly dependent upon the species and NaCl concentration. It was proposed that wild *Phaseolus* species and *P. filiformis*, in particular, represent a genetic resource for improvement of salt tolerance of common bean.

Salt tolerant genotypes of mulberry (*Morus* spp.) were identified through screening of the seeds under *in vitro* conditions. Seeds from different genotypes showed wide variability to salinity. Thus, these genotypes could be selected for further testing on saline soils under *ex vitro* conditions and offers an added scope of selecting the seedlings, which showed tolerance to salinity. These seedlings can be transplanted to field conditions through gradual hardening like other tissue-cultured plants. Since screening of large number of genotypes under *ex vitro* conditions entails huge investment and often the interaction of other soil factors make the assessment difficult, screening under *in vitro* conditions is an attractive alternative, which is more easy, efficient and needs less space and time to screen large number of seeds and genotypes for salinity tolerance (Vijayan et al., 2003).

Twenty-nine genotypes of Ethiopian mustard (*B. carinata*) were tested for their tolerance to salinity at seedling stage. Percent reduction in seedling growth parameter viz; percent seed germination, speed of germination, root length, shoots length and seedling dry weights were observed and seedling vigour was calculated. The percent reduction in seedling vigour was used as a preferential parameter over others for final screening purpose followed by percent reduction in seed germination, percent germination, respectively (Thakral et al., 2001). They advocated that genotypes that show more tolerance at seedling stage are likely to establish better in saline soils, and even though positive correlation may not exist between seedlings and adult stage, the germination of seeds itself is one of the most essential aspect and screening at seedling stage in extremely important. Between and within cultivars variability in salt-tolerance in Lucerne (*Medicago sativa L.)* was evaluated in two-week-old seedlings of 35 cultivars to increasing NaCl concentration by Al- Khatib et al., (1994). Shoot length was used as a criterion for assessing salt tolerance, it decreased significantly with increasing NaCl concentrations for all cultivars, but there was considerable variation in response between and within cultivars. They concluded that selection between and within cultivars should lead to increased salt tolerance in this species. Effects of salinity were investigated in two salt tolerant wheat varieties, Cieta Serros and Maxipak, at germination and seedling stages. Seeds were treated during germination with either distilled water or one of six concentrations of either NaCl or KCl. Both salts reduced final germination percentage, germination rate and increased the production of abnormal seedlings (i.e.., where radicle and/or plumule structures were deformed, missing or incomplete). Low concentrations of either salt (0.05, 0.01 M) had no significant effects on final germination percentage and only slight effects on germination rate. High concentrations (0.2-0.4 M) had significant effects on all three parameters. No germination occurred during a 'germination recovery' (where ungerminated seeds were transferred to distilled water for recovery for 10 days). They suggested that effects of NaCl and KCl may be due to ionic toxicity rather than osmotic inhibition (AL-Ansari, F.M, 2003).

Salt tolerance was evaluated in 340 accessions of *Hordeum* consisting of 41-brittle rachis forms of *Hordeum vulgare* subsp. *vulgare* (*H. agriocrithon)* accessions, 154 *H. vulgare* subsp. *spontaneum* accessions and 145 accessions of ten other species or subspecies of wild *Hordeum*. The levels of salt tolerance for seed germination in wild *Hordeum* species were generally lower than those previously found in cultivated barely. Leaf injury index was used to assess tolerance at the seedling stage after treatment with 500 mM NaCl

solution for 4 weeks. The levels of salt tolerance at the seedling stage in wild *Hordeum* species were generally higher than those found in cultivated barley. It was advocated that wild *Hordeum* species could be considered as good source of germplasm for salt tolerance breeding (Mano and Takeda, 1998). Heritability of salt tolerance in germinating seeds of barley was estimated by parent progeny correlation and selection responses. Germination tests in 250 mM NaCl solution were conducted using the seeds of F1, F2 and F3 plant derived from crosses between salt tolerant (OUJ 417) and (OUK 666) and sensitive (OUE 211) varieties. Salt tolerance in the 2 crosses showed polygenic segregation in the F2, F3 and F4 generations. Correlation coefficients between parents and off springs reaction in the crosses were about 0.5 between the F2-F3 generation realized heritability estimated from the ratio of selection/responses selection differential were 0.2-0.4 in F2 – F3 and 0.8 – 0.9 in F3 – F4 plants. Although dominant genetic variance in the F2 generation, heritability of this trait was relatively high even between the F3- F4 generations and higher heritability may be obtained in later generations (Mano and Takeda, 1997).

Chaudhary et al., (1995) advocated that simple measurement of electrical potential difference (PD) for plant growth in a given concentration of NaCl over a given period of time would provide a fairly rapid screening method for salt resistance in rice and possibly other species.

Effects of sodium chloride salinity on seed germination, seedling root and shoot extension growth of four *Sorghum bicolor* cultivars was evaluated by Macharia et al. (1995). They observed that seed germination, seedling root and shoot extension of 4 sorghum cultivars decreased with increasing salinity. Transfer of seeds that had not germinated in various salt treatments to distilled water, only slightly increased the number of seeds that germinated. The decrease in seed germination and shoot/root extension was attributed largely to ionic toxicity rather than to osmotic factors.

Mano et al., (1996) evaluated the varietal response and effects of some major genes on salt tolerance at the germination stage in barley. A total of 6712 barley varieties and 368 isogenic lines were germinated in 1, 1.5 and 2% NaCl solution to select for salt-tolerance. The findings suggested that *v*, *n* and *uz* genes affected salt tolerance. A high correlation between the reactions to NaCl and polyethylene glycol treatments revealed that the effect of salt-stress on germination was mainly due to osmotic stress. The varieties from China and Korea were more tolerant than varieties from Turkey and Japan. Salt tolerance at the germination stage was independent of the salt tolerance at the seedling stage.

In order to identify the degree of salinity tolerance of the indica and japonica rice groups, 10 varieties were tested under saline and non-saline conditions by Lee Kyu Seong et al., (2003). Twelve-day-old seedlings were grown in normal culture solutions, then salinized at an electrical conductivity (EC) of 6 ds/m for 4 days and finally salinized at an EC of 12 d s/m for the next 14 days. The growth parameters and Na^+ and K^+ absorption in the shoot were measured to characterize the tolerance level of the two groups. Reduction in all growth parameters of tolerant varieties was significantly lower in indica varieties than in japonica varieties. Tolerant indica varieties were good Na^+ excluders, absorbed high amount of K and maintained a low Na/K ratio in the shoot. These results indicated that the tolerance level of indica was higher than that of japonica.

Tolerance at one developmental stage is unreliable for predicting the tolerance at other stages of development. Screening schemes should therefore, involve assessment at specific development stages throughout the ontogeny of the plant. The first exposure of a crop to salinity stress occurs at the germinating stage and is likely to proceed further under higher surface soil salinity than would be the case for later growth stages (Bernstein and Hayward, 1958). Hence, improving the uniformity and rapidity of seed germination under salinity might contribute significantly to the efficiency of stand establishment. However, Foolad and Jones, (1993) advocated that salt tolerance at germination and at the seedling stage to be controlled by more than one gene and are highly influenced by salt concentrations. The evaluation of germplasm for salinity tolerance under field conditions is complicated by the heterogeneity in salt concentrations at different depths in the soil, time and space and by the different responses of plants to salt stress at different stages of growth. Usually empirical methods are used to screen germplasm lines for salt tolerance. Although salt tolerant lines have been identified in screening experiments for many crops. Development of broad based germplasm pools by intercrossing salt tolerant lines of diverse origin followed by mass propagation under highly saline conditions is a realistic approach. Maximum benefits from such salt tolerant germplasm pools could be derived by maintaining them in a dynamic state of genetic heterogeneity. Planned introgression from promising wild relatives should be allowed to enhance genetic diversity in these salt tolerant germplasm pools. These enriched and dynamic populations would then form the basic materials in breeding for salt tolerance (Jana, 1993).

MATERIALS
AND
METHODS

MATERIAL AND METHODS

The present work was carried out to see the response of different genotypes of *Trifolium alexandrinum* to varying levels of salinity under *in vitro* conditions. Different materials used in the study and methods applied are described below:

Materials

The study involved 34 genotypes of *T. alexandrinum* representing three different ecotypes procured from the Gene Bank of IGFRI, Jhansi. Details of genotypes are presented in table 2:

Methods

A. *In vitro* screening

A.1. Preparation of media: MS media (Murashige and Skoog, 1962) was used for the germination and *in vitro* seedling development. The inorganic salts were mixed in double distilled water supplemented with 0.3% sucrose and solidified by adding agar 0.7%. The prepared media was divided into 4 parts. The 3 parts were supplemented with 0.25%, 0.50% and 0.75% NaCl and the fourth part was used as normal (control). The pH of the control medium was adjusted to 5.8 whereas and its electrical conductivity (EC) in the 3 treatments was: 0.25 %(EC 4.8 dSm-1), 0.50 %(EC 8.1dSm-1) and at 0.75 %(EC11.3dSm-1). The prepared media (30 ml) was poured in each 250 ml conical flask and plugged with non-absorbent cotton wrapped in muslin cloth. The medium was autoclaved for 25 minutes at 15 Lbs/sq. inch pressure. The autoclaved medium was allowed to cool quickly and kept in dark. After the solidification at room temperature the medium was used for inoculation of seeds of different genotypes.

A.2. Inoculation of seeds and rising of plants: Healthy seeds of the respective genotypes were surface sterilized by immersing in 0.1% $HgCl_2$ for 1.5-2.0 minutes followed with 4-6 washings in sterile distilled water. Twenty surface sterilized seeds were inoculated in each conical flask in all the 3 salt supplemented MS media and normal MS medium. These were incubated in dark at 25 ± 2^0C till germination was initiated. After germination cool fluorescent light was provided for 8-10 hours per day.

A.3. Data recording: Data for germination of seeds was recorded every 5th day and for morphological characters on the 20th day after inoculation. The seedlings were removed from the media carefully so that the roots were not damaged, washed in cool water and

Table 2. Details of genotypes of *T. alexandrinum* used in the study.

S. No.	Ecotype	Ploidy	Original Name
1	Saidi	Diploid	EC 329299
2.	Fahli	Diploid	EC 318954
3.	Mescavi	Diploid	Wardan
4.	Mescavi	Diploid	EC 407709
5.	Mescavi	Diploid	EC 400976
6.	Mescavi	Diploid	EC 508311
7.	Mescavi	Diploid	EC 4017103
8.	Mescavi	Diploid	EC 400977
9.	Mescavi	Diploid	EC 401711
10.	Mescavi	Diploid	ISH 34/49
11.	Mescavi	Diploid	ISH 34/41
12.	Mescavi	Diploid	ISH 34/11
13.	Mescavi	Diploid	Penta 99
14.	Mescavi	Diploid	Raj Bundi
15.	Mescavi	Diploid	Penta 99-1
16.	Mescavi	Diploid	ES 99
17.	Mescavi	Diploid	ISH 32/8/1
18.	Mescavi	Diploid	Wardan S2
19.	Mescavi	Diploid	ISH 26/50/7
20.	Mescavi	Diploid	ISH 32/34/1
21.	Mescavi	Diploid	Multi 98-45
22.	Mescavi	Diploid	ISH 34/5/1
23.	Mescavi	Diploid	Raj 49/50
24.	Mescavi	Tetraploid	T 44-4
25.	Mescavi	Tetraploid	T 45-1
26.	Mescavi	Tetraploid	T 5-90I-1
27.	Mescavi	Diploid	ISH 8020B
28.	Mescavi	Diploid	ISH 8020 Y
29.	Mescavi	Diploid	ISH 5050 B
30.	Mescavi	Diploid	ISH 5050 Y
31.	Mescavi	Diploid	ISH 34/8 B
32.	Mescavi	Diploid	ISH 34/8 Y
33.	Mescavi	Tetraploid	T 5-90-I
34.	Mescavi	Tetraploid	T-9-90FM

data recorded for length of shoot, roots, number of leaves and weight of plant. The plants were thereafter stored at -5^0C for isozyme analysis. Data of six seedlings were recorded from each conical flask, three for susceptible type of plants and 3 for resistant type of plants. The plants were differentiated into susceptible and resistant plants primarily on the basis of root growth. The susceptible type of plants had abnormal pattern of growth of the roots i.e., the roots were aerial, hair –like and sometimes the development of root was totally absent whereas in the resistant plants the development of root was positively geotropic although reduced at different salinity treatments. In control flask data was recorded on six plants for the purpose of data analysis of susceptible and resistant set of plants.

In the second set of experiments seeds were inoculated in three replications at three salinity levels and control, allowed to grow for 45 days. Similar methodology was employed for data recording in 45-day old seedlings except that the differentiation between susceptible and resistant plants was not considered. As prolonged exposure to salinity conditions had a degenerating effect on the susceptible type of plants primarily due to negative growth pattern of roots and the plants degenerated by 45th days and mostly the resistant plants with positive roots survived.

A.4. Statistical analysis: Analysis of variance was done using MS Excel programmes. Percentage of reduction due to salinity stress in relation to non stressed conditions was also determined for all the traits observed. Salinity susceptibility index for germination, shoot length, root length, number of leaves and biomass of the plants under stress (0.75% salinity) was calculated as follows: SSI = (1 – Y_{ss}/Y_{ns})/SII, where Y_{ss} and Y_{ns} are the mean values of the traits for each genotype in maximum salinity and non-saline conditions respectively and SII= 1-(Average value of the trait of genotypes in stress condition/Average value of the trait of genotypes in non-stress condition) (Fisher and Maurer, 1978).

B. Callusing Response

Three ecotypes of *T. alexandrinum* i.e., Saidi (EC 329299), Fahli (EC 318954) and Mescavi (Wardan) were used for this study.

B.1. Preparation of culture media: Different combinations of inorganic media with varying levels of hormonal concentrations were used at different stages of callus culture.

L_2* *media: This media was used for callus induction and differentiation. Inorganic salts (Philips and Collins, 1984) were mixed in double distilled water. Growth regulators were

added in different concentrations for various purposes. The prepared media was divided into 4 parts. Three parts were supplemented with 0.25%, 0.50% and 0.75% NaCl respectively while the 4^{th} part was used as normal. The prepared media was poured into test tubes about 10-15 ml media per test tube and plugged. The medium was autoclaved and other procedures followed as mentioned before.

B.2. Explants: Explants were taken from 20-25 days old healthy plants grown under aseptic condition on MS normal medium. Plant parts used as explants were petiole and hypocotyls. 0.3- 0.4 cm long pieces were cultured on the callusing media.

B.3. Callus induction: Explants from healthy seedlings were inoculated aseptically in the culture tubes containing callus induction media. 2 explants were cultured in each tube, 5 tubes per genotype per explants (i.e., hypocotyls and petiole) for every treatment (i.e., 0.25%, 0.50%, 0.75% and normal L_2 media) were cultured.

The periodical observations were recorded for callus induction response in percentage on 7^{th}, 14^{th}, 21 and 28^{th} days for nature, colour and growth rate of callus. After 28 days the callus were split and sub–cultured in 10-20 tubes as per the availability of callus into callus inducing media of higher salinity level and regenerating media. Similar observations were recorded up till 28 days and after which the available callus was sub-cultured to further higher salinity containing callus inducing media, regenerating and shoot inducing media and observations recorded up to 28 days.

C. Embryo culture

Flower buds of the three ecotypes of *Trifolium* i.e., EC 329299 (Saidi), EC 318954 (Fahli) and Wardan (Mescavi) were brought to laboratory. Mature ovules were excised, surface sterilized using 0.1% $HgCl_2$ under aseptic condition and inoculated in suitable culture media.

C.1. Preparation of media: MS (Murashige and Skoog, 1962) inorganic media supplemented with 0.5 mg/l kinetin, 3% sucrose and 0.7% Agar was prepared in double distilled water. The pH of the media was adjusted to 5.8 with IN NaOH. After the addition of agar the medium was poured into test tubes and plugged with non-absorbent cotton wrapped in muslin cloth. The prepared media was divided into 4 parts. The three parts of the media were supplemented with 0.25%, 0.50% and 0.75% NaCl and one part without salt was taken as normal. The medium was autoclaved for 25 minutes at 15-psi pressure. Tubes containing autoclaved medium were left for overnight for solidification, the medium was used for embryo culture.

C.2. Data –recording: Two embryos were inoculated per tube, 25-30 tubes were cultured per genotype for every treatment i.e., 0.25%, 0.50%, 0.75% and control media. The cultures were maintained under standard conditions (25 ± 2^{0}C) under dark condition till embryo germinated. After embryo germinated the plants were provided with 16/8 hours of photoperiod.

Data for germination of ovules were recorded every day up to 7th day. Morphological data for plumule length, radicle length and number of leaves was recorded after 20th day of inoculation of embryo. Percentage mortality of seedlings was also recorded. After 20 days the surviving plants were transferred in MS media and data recorded after 20 days for similar morphological characters. The surviving plants were sub-cultured in RL (root inducing media). The RL media was divided into 3 parts, 2 parts were supplemented with 0.25% and 0.50% NaCl and the remaining was used as control. After 20 days of transfer, data was recorded for plumule length, radicle length, number of leaves and percentage mortality due to continuous exposure of plants in saline conditions. The surviving salt treated plants were finally transferred into RL media devoid of salt and data for morphological characters recorded. The plants were transferred to field condition.

C.3. Hardening and field transfer - Complete plants with root and shoot were hardened and transferred to field. The tubes were taken out of culture room and kept at room temperature for 2-3 days. The plantlets were than taken out of tube and washed carefully to remove the agar media. Extra care was taken at this stage so as to cause minimum damage to roots on shoots. The plants were transferred to pots having soil: sand ratio of 1:1. The pots were kept every day for 3-4 hours under field condition for 6-7 days. Extra care was taken to avoid desiccation of young leaves. Wrapping cellophane paper across the plant with sufficient holes for air transfer did this. After 6-7 days the plants were transferred to field conditions.

D. Pot culture studies

Eight different genotypes of *T. alexandrinum* representing Mescavi (EC 407709, EC 4017103, Wardan), Fahli (EC 318954), Saidi (EC 329299), tetraploid (T 45-1, T 5-90I-1) and interspecific (ISH 8020B) hybrid progeny were selected for biochemical and molecular studies in pot culture condition.

Healthy seeds of the respective genotypes were sown in clay pots (*kulhar*) filled with soil. 20 seeds of each genotype were sown in each pot. The pots were irrigated with 4 different concentrations of NaCl solutions i.e., 0.25% EC, 0.50%, 0.75%, 1% and plain water

respectively. The pots were irrigated daily with water supplemented with required amount of salt for saline treatments and with plain water for control. Samples for biochemical studies were collected on the 25th day after sowing.

D.1. Biochemical studies: Plants grown *in vitro* and in clay pots (*kulhar*) under different saline treatments as well as under control condition were analyzed using different biochemical parameters like isozyme, proteins, Na^+ and K^+ estimation and RAPD. PAGE polyacrylamide gel electrophoresis was used using polyacrylamide vertical gel electrophoresis system to study the isozymic and protein banding pattern of the plants growing under different salinity levels and under control condition.

Preparation of stock solutions, reagents and buffers

Acrylamide stock solution: - Acrylamide stock solution was prepared by dissolving 29.2 g Acrylamide and 0.8 g of bisacrylamide in double distilled de-ionized water and final volume was made up to 100 ml. Solution was stored at 4^0C in amber coloured bottle.

Tris HCl Buffer for separating (resolving gel) (pH 8.9): 18.15 g of Tris was dissolved in 60 ml of water and pH was adjusted to 8.9 by adding drops of 1N HCl and final volume was made up to 100 ml using de-ionized double distilled water.

Tris HCl buffer for stacking gel (pH 6.8) – 7.26 g of Tris was dissolved in 60 ml of water and pH was adjusted to 6.8 by adding drops of 1N HCl and final volume was made up to 100 ml using de-ionized double distilled water.

Ammonium per sulphate (APS) – 100 mg Ammonium per sulphate was dissolved in 100 ml distilled water. This solution was prepared fresh each time.

Running gel electrode buffer (pH 8.2) – Electrode buffer (pH 8.2) was prepared by dissolving 600 mg of Tris and 2.8 g of Glycine in 1 litre distilled water.

Tracking dye: Tracking dye was prepared by mixing 0.25% Bromophenol blue with 40% sucrose solution.

Grinding buffer – 605 mg of Tris was dissolved in 20 ml of distilled water after that 168 mg of EDTA (Ethylenediaminetetra acetic acid) was dissolved and 40 ml of distilled water added to dissolve 5.0 g of sucrose and 100 ml of Marcaptoethenol was added to the solution. Finally the volume was made up to 100 ml using de-ionized double distilled water.

Gel buffer (pH – 8.65) – Tris citrate buffer: 9.206 g Tris and 1.051 gm citric acid were dissolved in distilled water and final volume was made up to 1 liter.

Acetate buffer - (pH 5.6) 27.216 g of sodium acetate trihydrate and 2.6 ml of acetic acid was dissolved and volume made up to 1000 ml by using distilled water.

Phosphate buffer (pH. 6.0)

(a) **Mono basic** – 31.2 g of sodium dihydrogen orthophosphate dissolved in 1000 ml distilled water.

(b) **Dibasic** – 28.4 g of disodium hydrogen orthophosphate dissolved.

Preparation of resolving gel – 40 ml 10% resolving gel was prepared by adding Acrylamide (30%, 16 ml), Tris HCl (10 ml), H_2O (14 ml), TEMED (20 μl) and Ammonium per sulphate (10%, 200 μl). The gel solution was poured in vertical gel casting unit and left for one hour for setting in undisturbed condition. At the top of resolving gel about 5 ml of distilled water is poured so that the gel does not get dried.

Preparation of stacking gel – 10 ml of 5% stacking gel was prepared by adding Acrylamide (30%, 1.7 ml), Tris HCl (1.3 ml), H_2O (6.9 ml), TEMED (10μl) and APS (50μl). This gel solution was poured over the resolving gel after removing the top level of water. Pouring of stacking gel was immediately followed with placing of comb. This gel was left for 1 hour. The comb was removed and 50 micro liter of sample was mixed with 10 micro liter of tracking dye and loaded in wells.

Electrophoresis - The gel plate thus prepared was placed in 'Genei' vertical migration chamber. Running gel electrode buffer was poured into the migration chambers so that electrodes were completely dipped. The plate was connected with the buffer chamber. A constant current of 20 MA was given till the tracking dye crossed the stacking gel. Thereafter the current was increased to 40 MA till the tracking dye reached the bottom of the gel.

Staining of the gel – The gels were stained for peroxidase following the method given by Veech (1969), for Esterase and SOD following the protocol given by Wendel and Weeden (1989).

Peroxidase – 100 mg of benzidine was dissolved by heating in 100 ml of 0.2 M acetate buffer (pH 5.6). In 100 ml of benzidine solution, 2 ml of 3% hydrogen peroxide was added at the time of incubation of gel. After 10 minutes of incubation, blue bands appeared which turned brown later. The oxygen released in the reaction, which oxidizes Benzidine, stained the sites of peroxidase isozyme, a colourless compound to coloured one.

Esterase - The gel was incubated in 100 ml of 0.1 M phosphate buffer (pH 6.0) containing 32.5 mg of 1-napthyl acetate in 1 ml acetone and 50 mg of fast blue RR salt at

room temperature for 60 minutes. The site of esterase enzyme activity appeared as reddish brown to blackish bands on the gel.

Superoxide Dismutase - The gel was stained in 100 ml of Tris – HCl buffer (pH 8.65) in which Riboflavin (4 mg), EDTA (2 mg) and NBT (20 mg) were added. The gel was incubated in dark for 30 minutes and then exposed to intense light for 30 minutes until clear bands appeared.

Native Protein – The gel for protein bands was stained by dissolving 40 mg of Coomaise brilliant blue R-250 in 100 ml solution of 40ml methanol, 10 ml Acetic acid glacial and 50 ml distilled water. The gel was left overnight in the stain. The gel was put in a de-staining solution next day to remove the extra stain from the gel. The de-staining solution was prepared by adding 40 ml methanol, 10 ml acetic acid and 50 ml distilled water.

Preparation of Zymogram – The isozymic and protein bands were drawn on graph sheet at 1:1 ratio. The point of origin was marked in order to see the relative mobility of the bands. Selected gel plates were photographed.

Scoring and nomenclature of bands – The bands were scored from the starting point i.e., the well where the samples were loaded. The slowest band was treated as first band and the bands were numbered.

D.2. Discontinuous SDS polyacrylamide gel electrophoresis (SDS-PAGE) – SDS polyacrylamide gel electrophoresis was used to evaluate the protein profile response of 8 selected genotypes of *T. alexandrinum* under varying levels of NaCl concentration. The samples were collected from the plants growing in clay pots (*kulhar*) under 4 salinity treatments and control condition respectively. The samples were ground in pastel and mortar using 2x sample buffer under cold conditions. The samples were collected in eppendorf, kept at 37^0C overnight, boiled for 3-5 minutes and finally centrifuged at 10,000 rpm. The supernatant was poured in fresh eppendorf and stored.

Discontinuous SDS polyacrylamide gel electrophoresis (SDS–PAGE) was performed using a system based on that of Laemmli (1970) with minor modifications as required. Equal quantities of samples were loaded on 10% Acrylamide gel. The gel was run at 120 V in stacking followed by 200 V in resolving under cold conditions. After the dye reached the bottom, the gel was removed in 5% TCA (Trichloro acetic acid) solution for 30-45 minutes, followed by staining of gel in 5% of Coomasie brilliant blue R –250 containing 40% methanol, 10% acetic acid. Bands appeared after 24 hours. Excess of stain was removed by using de-staining solution. Protein bands were identified using molecular weight marker (PMW-B, Broad range) of 'Genie' make.

D.3. Flame Photometry: Na^+ and K^+ were estimated by the Digital Flame photometer using the method given by Jeffery (1989). The samples were collected from the plants growing in clay pots (*Kulhar*) under 4 salinity treatments and control condition. The plants were uprooted, washed with water and blotted dry. The shoot and root portion were separated. The plant parts were dried in oven at 80^0C for 4 days. The dry samples were ground into powder using sample grinder. The powdered samples were collected in polythene bags. 100 mg of dry sample was taken in 10 ml of perchloric acid ($HClO_4$) and kept over night. It was then digested at 200^0C for 2 hours initially followed by 300^0C till the samples turned colourless. The digested aliquot was poured in 50 ml volumetric flasks and final volume made 50 ml using double distilled water. The reading was noted from the Digital Flame Photometer.

E. Sand culture studies

Five selected genotypes of Berseem i.e., EC 318954, EC 329299, T 45-1, EC 407709 and ISH 8020B were used. Healthy seeds were germinated in pots containing washed sand moistened with water up to first leaf emergence (10 to 12 days after sowing); the pots were thereafter irrigated with nutrient solutions as given by (Shannon and Noble, 1995) supplemented with 0.50%, 0.75% and 1% NaCl. Seedlings were irrigated every day with 500 ml nutrient solution/pot. The sand of each pots washed every 5th day by irrigating it with running plain water for 5 minutes to prevent salt-build up. The morphological data were recorded on 60th day after sowing. Isozymic and protein banding pattern was observed in the leaves collected on 60th day.

F. DNA extraction and Polymerase Chain reaction PCR

Isolation of plant DNA – DNA was isolated from 500 mg of fresh leaves following the method of Iqbal et al., (1997) with suitable modifications. The leaves were ground in sufficient quantity of liquid N2 to avoid any nuclease activity and to obtain non-sheared DNA. Fine powder of the leaf extraction buffer (pH 8.0) [2% W/V CTAB, 100 mM Tris-Cl, 20 MM EDTA and 1.4 m NaCl) containing 1% marcapto-ethanol. This was uniformly mixed and incubated at 65^0C for 1 hour with gentle shaking. The mixture was emulsified with an equal volume of Chloroform-isoamyl alcohol. The slurry was centrifuged at 8000 rpm for 15 minutes. The upper aqueous phase was removed and nucleolus was precipitated with 0.6 volume of isopropanol. This was kept at -20^0C for an overnight. Next day it was centrifuged at 8000 rpm for 10 minutes and the supernatant discarded. The pallet obtained was air dried and dissolved in 500µl T.E buffer (pH 8.0). The solution

containing RNA and DNA was treated with RNAase (10 μ g/ml) and kept at 37^0C for 1 hour. Now the solution was extracted with phenol/chloroform (1:1) and finally with chloroform. This was centrifuged at 8000 rpm for 10 minutes; thereafter the upper aqueous phase was collected in fresh tubes. 1 ml of absolute ethanol was added to precipitate DNA. For complete precipitation of DNA it was left overnight at -20^0C. Next day centrifuging at 8000 rpm for 15 minutes precipitated the DNA. The supernatant was discarded and the DNA pallet was washed with 70% ethanol, it was again centrifuged at 8000 rpm for 10 minutes, the supernatant was discarded and the DNA pallet vacuum dried and finally dissolved in 500 μl of T.E. buffer. The concentration and quality of DNA was estimated by 0.8% agarose gel electrophoresis and with spectrophotometer taking OD at 260/280 nm.

Table 3. List of primer used

S. No.	Primer	5' to 3'	S. No.	Primer	5' to 3'
1	OPE-12	TTATCGCC	13	AB-10	GGACCCTTAC
2	OPF-6	GGGAATTCGG	14	B-5	TGCGCCCTTC
3	OPG-12	CAGCTCACGA	15	R-08	CCCGTTGCCT
4	OPH-9	TGTAGCTGGG	16	AK-14	ACCCGGAAAC
5	OPQ-3	GGTCACCTCA	17	U-01	ACGGACGTCA
6	OPN-6	GAGACGCACA	18	P-9	GTGGTCCGCA
7	AE-01	TGAGGGCCGT	19	H-15	ACTGGGACTC
8	AE-03	CATAGAGCGG	20	E-16	GGTGACTGTG
9	AH-9	AGAACCGAGG	21	B-14	GGAGGGTGTT
10	OPQ-06	GAGCGCCTTG	22	V-02	AGTCACTCCC
11	OPR-06	GTCTACGGCA	23	N-20	GGTGCTCCGT
12	AB-05	CCCGAAGCGA			

Polymerase chain reaction and RAPD analysis – Polymerase chain reaction was carried out in a 20μl solution containing 1x Taq Polymerase buffer, 2.5 mM each of dATP, dGTP, dCTP, dTTP, 0.5 unit of Taq DNA Polymerase (Bangalore, Genei, India), 25 ng of template DNA and 20 ng of primer (Operon, Inc. U.S.A). The reaction mixture was overlaid with one drop of mineral oil in order to avoid evaporation. The amplifications were carried out in MJ Research PTC–200 peltier thermal cycler,

programmed for 40 cycles of 94^0C for one minutes (denaturation) 37^0C for one min (annealing) and 72^0C for two min (amplification). After completion of 40 cycles, the reaction was kept on 72^0C for 10 min and than held at 4^0C until the tables were removed.

Agarose gel electrophoresis of PCR product – The PCR Product were separated on 1.6% agarose with ethidium bromide in the gel using 0.5 x Tris borate EDTA (TBE) buffer. The reaction mixture was mixed with 2µl 10 x DNA loading Buffer and were loaded on the agarose gel. 100 base pair ladders were also loaded to identify the size of the amplified products. The gel was run at 70 volts for four hours. The amplified product was visualized under UV Trans illuminator and was photographed using Polaroid and SLR Camera.

Solutions and buffers for Plant DNA isolation

1. O.5 M EDTA pH 8.0: Dissolve 37.22 g EDTA disodium salt and 4.0 g sodium hydroxide in 150 ml DDW and pH adjusted by NaOH solution, final volume was made 200 ml.

2. 1M Tris-Cl pH (8.0): Dissolve 24.23 g Tris in 150 ml distilled water. HCl and final volume made 200 ml adjusted the pH to 8.0.

3. 5 M NaCl: Dissolve 58.44 g NaCl in distilled water and final volume adjusted to 200 ml.

4. CTAB (10% W/V): Dissolve 200 g CTAB in 150 ml distilled water CTAB was dissolved by warming the solution. The final volume was made up to 200 ml.

5. T.E. Buffer (pH. 8.0): 2 ml Tris-Cl from 1 M Tris-Cl Stock and 0.04 ml EDTA from 0.5 M EDTA stock. The final volume was made up to 200 ml.

6. Tris borate EDTA Buffer on TBE buffer (pH 8.0) : 27 g of Tris base, 13.75 g boric acid were dissolved in 400 ml distilled water and 10 ml (0.5 M) EDTA was added. Finally the volume was made up to 500 ml.

7. CTAB Genomic DNA Extraction Buffer was prepared:

8 ml (0.5 M EDTA), 20 ml (1 M Tris-Cl pH 8.0), 56 ml (5 M NaCl) and 40 ml (10% CTAB) were mixed to prepare buffer, of which 2.0 ml taken for 1.00 gram sample.

8. Loading dye: 10 ml of 0.5 M EDTA was dissolved in 20 g of sucrose and 125 mg Bromophenol blue. The final volume was made 50 ml with distilled water.

9. 10 X Taq buffer

10 mM Tris-Cl (pH 9.0), 15 mM $MgCl_2$, 50 mM KCl, 0.01% gelatin were mixed and final volume prepared in 100ml vile.

Cluster analysis: 216 fragments were used to generate the input matrix using computer software NTSYS pc 2.02e using SIMQUAL, similarity coefficients (DICE) was generated among 8 genotypes of Berseem. The DICE coefficient was used for SAHN clustering by UPGMA (Unweighted Pair Group Method with Arithmetical Averages). The Cluster analysis based on these values was used to generate a dendogram.

Table 4. Composition of L2, MS and RL basal media used in present study.

S N	Components	L2 basal	MS basal	RL basal
1	KNO_3	20.8 mM	18.8 mM	10.4 mM
2	NH_4NO_3	12.5 mM	20.6 mM	6.25 mM
3	KH_2PO_4	2.34 mM	1.25 mM	2.34 mM
4	$MgSO_4.7H_2O$	1.8 mM	1.5 mM	0.9 mM
5	$CaCl_2.2H_2O$	4.1 mM	3.0 mM	2.0 mM
6	NaH_2PO_4	0.6 mM	-	0.3 mM
7	$FeSO_4$.EDTA. 7 H_2O	90 µM	100 µM	90 µM
8	Na_2 EDTA. $2H_2O$	-	100 µM	-
9	$MnSO_4$. 4 H_2O	90 µM	100.0 µM	45 µM
10	H_3BO_3	82 µM	100.0 µM	41 µM
11	$ZnSO_4.7H_2O$	18 µM	30.0 µM	9 µM
12	KI	6 µM	5.0 µM	3 µM
13	$Na_2MoO_4.2H_2O$	1.7 µM	1.03 µM	0.85 µM
14	$CoCl_2.6H_2O$	0.42 µM	0.105 µM	0.21 µM
15	$CuSO_4$. 5 H_2O	0.4 µM	0.1 µM	0.2 µM
16	Myo-inositol	1.4 mM	100 mg/L	0.7 mM
17	Thiamine HCl	6 µM	0.1 mg/L	3.0 µM
18	Pyridoxine HCl	2.4 µM	0.5 mg/L	1.2 µM
19	Nicotinic acid	-	0.5 mg/L	8.5 µM
20	3-Aminopyridine	-	-	24 µM
21	Sucrose	73 mM	87.6 mM	44 µM
22	Agar	0.8 %	0.7%	0.65 %
23	pH	5.8	5.8	5.8

Table 5. **Composition of various media used in the present study.**

Media	Basal media	Auxin (mg/L)		Cytokinin (mg/L)	Auxin : Cytokinin
		NAA	**Picloram**	**BAP**	
Seedling raising media					
MS	MS	-	-	-	-
Callus inducing media					
	L2	5.00	-	1.00	5 : 1
Shoot inducing media					
	L2	0.0008	-	0.150	0.0053 : 1
Somatic embryogenesis media					
		2,4 – D		**Adenine**	
SEIM	L2	0.001	-	3.225	0.0003 : 1
Root inducing media					
		IAA			
RL	RL	0.21	-	-	-
Embryo culture media					
				Kinetin	
EC 3	MS	-	-	0.5	-

Table 6. Details of important chemicals used in the study.

Chemical	Make	Catalogue number	Molecular weight
1- Napthyl acetate	Hi Media	RM 1730	186.21
Fast Blue RR	Sigma	F-0500	387.9
NBT	SRL	144928	817.65
Riboflavin	Hi Media	RM 181	376.37
EDTA	Hi Media	RM 678	292.25
α ketoglutaric acid	Hi Media,	RM 245	146.1
L Aspartic acid	Hi Media	RM 083	33.10
PVP-40	SRL	164798	40,000
EDTA, Na_2 salt	Qualigens	18454101	372.24
Sodium phosphate dibasic	Loba Chemie	5972	141.96
Hydrogen peroxide solution	Qualigens	18755	34.01
Sodium 1- Napthyl phosphate	Loba Chemie	5945	264.15
Tris	SRL	2044122	121.14
N-N-N-N- Tetramethyl ethylendiamine (TEMED)	Loba Chemie	6230	116.21
Acrylamide	SRL	014022	71.08
N-N Methylene Bis acrylamide	Loba Chemie	4640	154.17
Ammonium Per Sulfate	SRL	0148134	228.20
Citric acid	Loba Chemie		192.13
Boric acid	Loba Chemie	43118	61.83
NaOH	Qualigens	27815	40.0
Sodium acetate trihydrate	Loba Chemie	46174	136.08
Glacial acetic acid	Qualigens	21057	60.05
Sodium dihydrogen orthophosphate	Hi Media	RM256	156.01
Disodium hydrogen Orthophosphate	Loba Chemie	45675	141.96
Adenine	Hi Media	RM 281	135.13
6-BAP	Hi Media	RM 787	
NAA	Hi Media	RM 575	186.21
Picloram	Sigma	P-5575	242.5
Kinetin	Hi Media	RM 448	215.21
IAA	Hi Media	RM 384	175.19
Agar	Hi Media	RM 666	
Mercuric chloride	Hi Media	RM 1383	271.5
Perchloric acid	Qualigens	29927	100.46
Coomasie Brilliant blue R-250	Hi Media	RM 344	826.0
Taq DNA Polymerase	Genei	MME24S	
dNTP	Genei	FC10L	
Agarose	Genei	AG2	

RESULTS

RESULTS

The experiments of the present work are grouped in following heads:

A. *In vitro* seedling vigour under saline vis-à-vis normal condition.
B. *In vitro* plant growth under saline vis-à-vis normal condition.
C. Isozyme studies in seedlings growing *in vitro* under saline vis-à-vis normal condition.
D. Biochemical studies in seedlings growing in pots under saline vis-à-vis normal condition.
E. Effect of secondary salinization on selected genotypes in sand culture.
F. *In vitro* callusing response under saline vis-à-vis normal condition.
G. *In vitro* embryo culture response under saline vis-à-vis normal condition.
H. Molecular characterization of selected genotypes.

A. *In vitro* seedling vigour under saline vis-à-vis normal condition

In vitro seedling vigour was observed among 34 genotypes and data recorded on germination, shoot length, root length, number of leaves, biomass and is presented in Tables 7-10 and Fig 2 to 6.

A.1. Germination, biomass and morphological observations

The results are being presented genotype wise.

EC 329299

Germination: During the initial period of exposure to salinity i.e. by 10^{th} day the germination was 51.66%, 28.33%, 0% and 93.3% at 0.25%, 0.50%, 0.75% and control condition respectively. By 20^{th} day the rate of germination was 90%, 68.3%, 26.7%, and 94.3% at 0.25%, 0.50%, 0.75% and control condition respectively. Thus, higher levels of salinity not only reduced the germination but also delayed the process.

Shoot length: The growth and development of shoot was highly inhibited at higher salinity level in the susceptible type of plants whereas in the resistant type of plants the inhibitory effect of salt stress was more visible at 0.75% salinity. In the susceptible type of plant at 0.25% salinity the average shoot length was 5.55 cm, at 0.50% it was drastically reduced to 0.80 cm, at 0.75% it was 0.96 cm. In the resistant type of seedlings at 0.25% the average shoot length was 5.94 cm, at 0.50% it was significantly reduced to 3.25 cm, at 0.75% it was further reduced to 1.34 cm and under control condition the average shoot length of the seedlings was 7.01 cm. Thus increasing salinity level reduced

the growth of the seedlings but at 0.75% salinity the inhibitory effect was more manifested as compared to the preceding two salt treatments and control.

Root length: In the susceptible type of seedlings at 0.25% the average root length was 5.14 cm, at 0.50% it was drastically reduced to 1.47 cm, at 0.75% the growth of roots was almost completely inhibited to 0.27 cm and roots were aerial, hair like negatively geotropic. In the resistant type of seedlings at 0.25% the average root length was 5.76 cm, at 0.50% it was significantly reduced to 2.45 cm, at 0.75% it was highly inhibited to 0.98 cm, and under control condition the average root length was 6.41 cm.

Number of leaves: In the susceptible type of seedlings at 0.25% the average number of leaves was 3.50, at 0.50% it was reduced to 2.00, at 0.75% it was further reduced to just 1.18. The growth and development of leaves in the tolerant type of seedlings at 0.25% was at par with the control plants whereas at 0.50% and 0.75% the growth of leaves was inhibited. In the resistant type of seedlings at 0.25% the average number of leaves was 3.74, at 0.50% it was reduced to 2.60, at 0.75% it was significantly reduced to 1.39 and under control condition it was 4.13. Thus a gradual decrease in the number of leaves was observed as the level of salinity was increased.

Weight of plant: In the susceptible type of seedlings at 0.25% salinity the average biomass production was 144 mg, at 0.50% it drastically reduced to 44 mg, at 0.75% it further reduced to 29 mg and under control condition the average biomass production of the seedlings was 132 mg. In the resistant type of seedlings at 0.25% salinity the average biomass production was 152 mg, at 0.50% it was significantly reduced to 90 mg, at 0.75% it was further reduced to 40 mg thus, low level of salinity i.e. 0.25% increased the biomass production in the growing seedlings and further increase in the salinity level had a deleterious effect.

EC 318954

Germination: During the initial period of exposure to saline conditions i.e. by 10th day the germination was 38.3%, 45%, 3.3% and 91.6% at 0.25%, 0.50%, 0.75% salinity level and control condition respectively. By the 20th day the rate of germination was 90%, 86.7%, 38.3%, and 96.0% at 0.25%, 0.50%, 0.75% salinity level and control respectively indicating that germination under salt stress was delayed initially but picked up and was at par to the control conditions at 0.25% and 0.50% salinity.

Shoot length: In the susceptible type of plant at 0.25% the average shoot length was 5.58 cm, at 0.50% it was significantly inhibited to 1.59 cm, at 0.75% it was further reduced to 1.06 cm. In the resistant type of seedlings at 0.25% the average shoot length was 5.95 cm,

at 0.50% it was reduced to 3.04 cm, at 0.75% it was significantly reduced to 1.66 cm and under control condition it was 7.50 cm.

Root length: In the susceptible type of seedlings at 0.25% the average root length was 5.36 cm, at 0.50% it was almost completely inhibited to 0.34 cm, at 0.75% it was only 0.11 cm and the roots were aerial, hair like and negatively geotropic. In the tolerant type of seedlings at 0.25% the average root length was 6.13 cm, at 0.50% it was significantly reduced to 2.98 cm, at 0.75% it was highly inhibited to 0.84 cm and under control condition the average root length was 7.49 cm. Thus, higher salinity level i.e. 0.50% and 0.75% had inhibited the growth and development of roots.

Number of leaves: In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 3.21, at 0.50% it was substantially inhibited to 1.07, at 0.75% it was 1.07. In the tolerant type of seedlings at 0.25% salinity the average number of leaves was 3.20 at 0.50% it reduced to 1.85, at 0.75% it further reduced to 1.39 and under control conditions it was 4.13. Thus, a gradual decline in the average number of leaves was observed with increasing salinity level.

Weight of plants: In the susceptible type of seedlings at 0.25% salinity the average biomass production was 109 mg, at 0.50% it declined sharply to 47 mg, at 0.75% it was 44 gm. In the resistant type of seedlings at 0.25% salinity the average biomass production was 118 mg, at 0.50% it significantly decreased to 68 mg and at 0.75% increase in the biomass production of seedlings was observed which was 186 mg and under control condition the average biomass production was 217 mg. Thus, the biomass production at 0.75% salinity was at par with control. These results indicated the tolerance level of this genotype to salt stress conditions.

Wardan

Germination: Germination during the initial period of exposure to salinity i.e. by the 10th day was 10%, 18.3%, 0% and 20% at 0.25%, 0.50%, 0.75% salinity level and control condition respectively. By 20th day the germination was 66.7%, 36.7%, 18.3%, and 75% at 0.25%, 0.50%, 0.75% salinity level and control condition respectively.

Shoot length: In the susceptible type of seedlings at 0.25% salinity the average shoot length was 0.83 cm, at 0.50% it was 0.83 cm, at 0.75% it was 0.85 cm. In the resistant type of seedlings at 0.25% salinity the average shoot length was 2.55 cm, at 0.50% it was reduced to 1.45 cm, at 0.75% it was substantially reduced to 0.90 cm and under control condition the average shoot length was 3.76 cm.

Root length: In the susceptible type of seedlings at 0.25% the average root length was 0.91 cm, at 0.50% it was 0.34 cm, at 0.75% it was 0.28 cm. The growth and development of roots was inhibited under salt stress and the roots were aerial, hair like and negatively geotropic. In the resistant type of seedlings at 0.25% salinity the average root length was 2.21 cm, at 0.50% it was drastically inhibited to 0.72 cm, at 0.75% it was further reduced to 0.27 cm, and under control condition the average root length was 2.97 cm. Thus the growth and development of root was highly inhibited at 0.50% and 0.75% salinity as compared to control and 0.25% salinity.

Number of leaves: In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 1.29, at 0.50% it was 1.18, at 0.75% it was 1.07. In the resistant type of seedlings at 0.25% the average number of leaves was 1.71, at 0.50% it was reduced to 1.25, at 0.75% it was further reduced to 1.07 and under control conditions the average number of leaves was 2.35. Thus, increasing levels of salinity inhibited the growth and development of leaves as compared to control.

Weight of plant: In the susceptible type of seedlings at 0.25% the average biomass production was 38 mg, at 0.50% it was 32 mg, at 0.75% it was 26 mg. In the resistant type of seedlings at 0.25% the average biomass production was 62 mg, at 0.50% it was 37 mg, at 0.75% it was 25 mg and under control condition the average biomass production was 68 mg.

EC 407709

Germination: Germination was reduced with increasing level of salinity but the reduction was marginal. During the initial period of exposure to saline conditions i.e. by 10^{th} day the germination was 56.6%, 67.5%, 31.6% and 95% respectively at 0.25%, 0.50%, 0.75% and control condition. The germination by 20^{th} day was 83.3%, 81.7%, 75%, and 95% at 0.25%, 0.50%, 0.75% and control condition respectively.

Shoot length: The average shoot length of the susceptible type of seedlings at 0.25% was 3.45 cm; at 0.50% it marginally increased to 4.17 cm, at 0.75% it was reduced to 2.58 cm. In the resistant type of seedlings at 0.25% the average shoot length was 8.84 cm, at 0.50% it was increased to 11.36 cm, at 0.75% it was marginally reduced to 10.72 cm as compared to 0.50% salinity but was more than at 0.25% salinity. Thus, the growth of seedlings at different salinity levels was comparable to the seedlings under control condition. This indicates the tolerance level of this genotype to saline conditions.

Root length: In the susceptible type of seedlings at 0.25% salinity the average root length was 1.89 cm, at 0.50% it increased to 2.88 cm, at 0.75% it was 2.81 cm. The growth of

roots was aerial, hair like and negatively geotropic. In the resistant type of seedlings at 0.25% salinity the average root length was 4.08 cm, at 0.50% it was slightly increased to 4.69 cm, at 0.75% it was reduced to 3.95 cm, and under control condition the average root length of the seedlings was 5.47 cm.

Number of leaves: The growth and development of leaves was substantially inhibited in the plants under salt stress conditions than the plants, which showed tolerant nature. In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 2.86, at 0.50% it was 2.14, at 0.75% it was 2.14. In the resistant type of seedlings at 0.25% the average number of leaves was 5.42, at 0.50% it was 4.96, at 0.75% it was 5.81 and under control conditions the average number of leaves was 6.00.

Weight of plant: The average biomass production in the susceptible type of plants was 116 mg, 69 mg, and 55 mg respectively at 0.25%, 0.50% and 0.75% salinity level. In the resistant type of plants the average biomass production under control condition was 161 mg, which was reduced in 0.25% to 107 mg, at 0.50% and 0.75% it was 161 mg and 142 mg respectively. Thus, higher levels of salinity had no inhibitory effect on the biomass production.

EC 400976

Germination: During the initial period of exposure to salinity i.e. by 10th day the germination was 15%, 0%, and 0% at 0.25%, 0.50% and 0.75% respectively, whereas under control conditions it was 87.5%. During the later period i.e. by 20th day the germination was 76.7%, 50%, and 13.3% at 0.25%, 0.50% and 0.75% salinity respectively whereas under control condition it was 90%. Thus, germination was reduced and delayed with increasing levels of salinity.

Shoot length: The average shoot length in the susceptible type of plants was 2.28 cm, 1.64 cm and 1.39 cm at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant group of plant the average shoot length at 0.25% was 6.25 cm, but was substantially reduced to 2.51 cm, and 1.51 cm respectively at 0.50% and 0.75% salinity as compared to 8.21 cm in control.

Root length: The average root length of the susceptible seedlings was 2.68 cm, 2.29 cm and 1.63 cm at 0.25%, 0.50% and 0.75% salinity. The roots were aerial, hair like and negatively geotropic. In the resistant type of seedlings under control condition the average root length was 7.11 cm, which was reduced to 4.45 cm, 2.76 and 1.56 cm respectively at 0.25%, 0.50% and 0.75% salinity.

Number of leaves: The average number of leaves in the susceptible type of plants was 2.36, 2.14 and 2.14 respectively at 0.25%, 0.50% and 0.75% salinity levels. In the seedlings growing under control condition the average number of leaves were 5.61, which were very marginally reduced among resistant types growing under stress condition to 5.35 at 0.25% salinity. As the level of salinity increased it reduced to 3.92 and 1.96 respectively at 0.50% and 0.75% salinity.

Weight of plant: The average biomass production of the susceptible seedlings was 60 mg, 37 mg and 42 mg at 0.25%, 0.50% and 0.75% salinity levels. In the resistant type of seedlings growing under control condition the average biomass production was 166 mg, which marginally reduced to 151 mg and 112 mg at 0.25% and 0.50% salinity respectively but at 0.75% the biomass production was significantly reduced to 49 mg.

EC 508311

Germination: By 10th day of inoculation the germination was 2.5%, 0%, 0 and 53.3% at 0.25%, 0.50%, 0.75% and control respectively. During the later period i.e. by 20th day the rate of germination was 16.7%, 11% and 0% at 0.25%, 0.50% and 0.75% respectively, whereas under control condition it was 70%.

Shoot length: The average shoot length of the susceptible type of seedlings was 2.21 cm and 1.78 cm at 0.25% and 0.50% salinity level respectively. In the resistant type of seedlings growing under control condition the average shoot length was 5.20 cm, which was reduced to 2.20 cm and 1.92 cm at 0.25% and 0.50% salinity respectively. No seedling was available for observation at 0.75%.

Root length: The average root length of the susceptible type of seedlings at 0.25% salinity was 1.28 cm whereas at 0.50% it increased to 2.11 cm, however, at 0.75% no samples were available for observation. In the seedlings growing under control conditions the average root length was 2.94 cm, which was reduced among resistant type of seedling growing under stress to 1.43 cm at 0.25% salinity treatment whereas at 0.50% salinity it increased to 2.08.

Number of leaves: The average number of leaves in the susceptible type of plants was 2.14 in both 0.25% and 0.50% salinity levels. In the resistant type of seedlings growing under control condition the average number of leaves were 3.24, which were reduced to 1.96 in both 0.25% and 0.50% salinity levels. No seedlings were available for observation at 0.75% salinity.

Weight of plant: The average biomass production in the susceptible type of plants decreased to 37 mg at 0.25% salinity whereas under 0.50% salinity condition the biomass

production increased to 42 mg. In the seedlings growing under control condition the average biomass production was 73 mg, which decreased in resistant type of plants growing under stress to 39 mg in both 0.25% and 0.50% salinity levels. No seedlings were available for observation at 0.75% salinity.

EC 4017103

Germination: During the initial period of exposure to salinity i.e. by 10^{th} day the germination was 13.3%, 3.3%, and 1.6% under 0.25%, 0.50% and 0.75% salinity respectively, whereas under control conditions 73.3% germination was observed. During the later period i.e. by 20^{th} day the germination was 28.3%, 13.3%, and 8.3% at 0.25%, 0.50% and 0.75% salinity respectively whereas under control condition 57.3% of the seeds germinated.

Shoot length: The average shoot length of the susceptible type of plants was 2.26 cm, 1.14 cm and 1.09 cm at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of seedlings under control conditions the average shoot length was 4.83 cm, which reduced to 2.28 cm, 1.14 cm and 1.26 cm at 0.50% and 0.75% salinity respectively.

Root length: The average root length of the susceptible type of seedlings was 1.09 cm at 0.25% salinity whereas under 0.50% and 0.75% salinity root growth was highly inhibited to 0.44 cm and 0.48 cm respectively. Roots were aerial, hair like and negatively geotropic. In the resistant type of seedlings growing under control condition the average root length was 2.20 cm, which was reduced to 1.08 cm at 0.25% whereas at higher salinity level i.e. at 0.50% and 0.75% the root growth was highly inhibited to 0.48 cm and 0.52 cm respectively but the roots had positive geotropic growth.

Number of leaves: The average number of leaves in the susceptible type of plants was 1.65 in all the three saline treatments. In the resistant type of plants growing under control conditions the average number of leaves was 2.78, which was reduced to 1.72 in all three saline treatments. Thus, there was no difference in response to salinity levels with regard to leaf emergence.

Weight of plant: The average biomass production in the susceptible type of plants was 45 mg, 39 mg and 26 mg at 0.25%, 0.50% and 0.75% salinity level. In the resistant type of seedlings growing under control condition the average biomass production was 121 mg which was very significantly reduced to 61 mg, 53 mg and 30 mg respectively at 0.25%, 0.50% and 0.75% salinity levels.

EC 400977

Germination: During the initial period of exposure to salinity i.e. by the 10th day the germination was 11.6, 0, and 0% at 0.25%, 0.50% and 0.75% salinity respectively, whereas under control condition the germination was 75%. During the later period of exposure to salinity the germination was 51.7%, 8.3% and 10% at 0.25%, 0.50% and 0.75% respectively and under control conditions 51.7% of the seeds germinated.

Shoot length: The average shoot length of the susceptible type of seedlings reduced to 1.62 cm, 1.03 cm and 1.43 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant type of seedlings growing under control condition the average shoot length was 4.46 cm which was reduced to 1.80 cm, 0.95 cm and 1.54 cm at 0.25%, 0.50% and 0.75% salinity level.

Root length: The average root length of the susceptible seedlings was 1.18 cm, 0.64 cm and 0.95 cm at 0.25%, 0.50% and 0.75% salinity level respectively. The roots were aerial, hair- like and negatively geotropic. In the seedlings growing under control condition the average root length was 2.40 cm which was reduced in resistant type of seedlings growing under stress to 1.22 cm, 0.63 cm and 0.90 cm at 0.25%, 0.50% and 0.75% salinity respectively. The growth of roots was inhibited but they were positively geotropic.

Number of leaves: The average number of leaves in the susceptible type of plants was 1.65 cm in all the saline treatments. In the resistant type of seedlings growing under control condition the average number of leaves was 2.23 cm, which was reduced marginally to 1.72 in all the saline treatments.

Weight of plants: The average biomass production in the susceptible type of plants was 32 mg, 32 mg and 19 mg at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant group of plants growing under control condition the average biomass production was 68 mg which was reduced to 38 mg, 38 mg and 30 mg at 0.25%, 0.50% and 0.75% salinity respectively.

EC 401711

Germination: During the initial period of exposure to salinity i.e. by the 10th day the germination was 20%, 6.6%, and 0% at 0.25%, 0.50% and 0.75% salinity level. During the later period i.e. by 20th day the germination was 36.7%, 16.7% and 8.7% under 0.25%, 0.50% and 0.75% salinity respectively whereas under control conditions 56.3% of the seeds germinated by the 20th day.

Shoot length: The average shoot length of the susceptible type of seedlings was 1.73 cm, 1.87 cm and 0.67 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the plants

growing under control condition the average shoot length was 6.29 cm which was reduced to 4.19 cm, 3.92 cm and 0.80 cm at 0.25%, 0.50% and 0.75% salinity level in the tolerant group of plants.

Root length: The average root length of the susceptible type of plants was 1.17 cm, 1.03 cm and 0.68 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the plants growing under control condition the average root length was 4.23 cm which was reduced to 2.44 cm, 2.18 cm and 0.69 cm at salinity level 0.25%, 0.50% and 0.75% respectively among resistant type of plants.

Number of leaves: The average number of leaves in the susceptible type of plants was 1.65 cm in all three saline treatments. In the seedlings growing under control condition the average number of leaves was 2.26 cm, which at 0.25% was increased to 2.49 whereas at 0.50% and 0.75% salinity it was decreased to 1.72 respectively among resistant type of plants.

Weight of plants: The average biomass production in the susceptible type of plants at 0.25%, 0.50% and 0.75 was reduced to 58 mg, 58 mg and 26 mg respectively. In the seedlings growing under control condition the average biomass production was 128 mg, which remained static at 0.25% salinity whereas at 0.50% it was marginally reduced to 114 mg and at 0.75% it was significantly reduced to 30 mg among resistant type of plants.

ISH 34/49

Germination: During the initial period of exposure to salinity i.e. by 10th day the germination under control condition was 95%, which was 73.3%, 26.6% and 10% at 0.25%, 0.50% and 0.75% salinity respectively. However, by 20th day the germination was 90%, 70% and 33.3% at 0.25%, 0.50% and 0.75% salinity respectively, whereas at control condition it remained 95%.

Shoot length: The average shoot length of the susceptible type seedlings was significantly reduced to 1.76 cm at 0.25% whereas at 0.50% it increased marginally compared to 0.25% salinity to 2.03 cm and at 0.75% it again reduced to 1.49 cm. In the resistant type of seedlings at 0.25% salinity average shoot length was 3.60 cm, at 0.50% and 0.75% salinity it was observed to be 2.43 and 2.72 cm respectively whereas under control condition it was 4.06 cm

Root length: The average root length of the susceptible type of seedlings was significantly reduced to 0.72 cm, 0.90 cm and 0.72 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant type of seedlings growing under control

condition the average root length was 2.54 cm which marginally reduced to 2.02 cm and 1.75 cm at 0.25% and 0.50% salinity whereas at 0.75% it reduced to 1.15 cm.

Number of leaves: The average number of leaves in the seedlings was reduced to 0.86 cm at 0.25% salinity whereas at 0.50% salinity it increased to 1.31 and at 0.75% it again decreased to 0.94. In the resistant type of seedlings growing under control condition the average number of leaves were 2.14, which reduced marginally to 1.64, 1.58 and 2.02 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of Plants: The average biomass production of the susceptible type of seedlings under saline conditions was 38 mg, 38 mg and 35 mg at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of seedlings growing under control conditions the average biomass production was 43 mg, which increased marginally to 47 mg both at 0.25% and 0.50% salinity level whereas at 0.75% salinity it was 40 mg.

ISH 34/41

Germination: During the initial period of exposure to salinity i.e. by 10th day, the germination under control was 90%, which was reduced to 56.6%, 26.6% and 33.3% at 0.25%, 0.50% and 0.75% salinity level respectively. However, by 20th day the germination was 56.7%, 46.7% and 43.33% at 0.25%, 0.50% and 0.75% salinity level respectively compared to 90% in control.

Shoot length: The average shoot length of the susceptible seedlings was 3.19, 1.99 and 1.10 cm at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of seedlings growing under control condition the average shoot length of the seedlings was 4.05 cm, which was reduced to 2.72 cm, 1.52 and 0.90 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the susceptible type of seedlings was 0.54, 0.36 and 0.31 cm at 0.25%, 0.50% and 0.75% salinity respectively. The roots had abnormal growth pattern i.e. the roots were aerial, hair-like and negatively geotropic. In the resistant type of seedlings growing under control condition the average root length was 2.35 cm which was highly inhibited to 0.60, 0.38 and 0.43 cm at 0.25%, 0.50% and 0.75% salinity levels respectively. The root growth was inhibited but the growth was positively geotropic.

Number of leaves: The average number of leaves in the susceptible type of plants at 0.25% salinity was 2.80 whereas at 0.50% and 0.75% it was reduced to 1.20 and 0.86 respectively. In the resistant type of seedlings growing under control condition the

average number of leaves was 2.62 which reduced gradually to 1.99, 1.25 and 0.98 from 0.25% to 0.75% salinity levels.

Weight of plants: The average biomass production in the susceptible type of seedlings at 0.25% salinity level was 71 mg whereas at 0.50% and 0.75% salinity it was reduced to 56 mg and 35 mg respectively. In the resistant type of seedlings growing under control condition the average biomass production was 84 mg which was reduced to 43 mg, 53 mg and 37 mg at 0.25%, 0.50% and 0.75% salinity respectively.

ISH 34/11

Germination: Germination under control condition by the 10th day was 93.3% whereas it was 95%, 70% and 41.6% at 0.25%, 0.50% and 0.75% salinity level respectively. By the 20th day the germination was 95%, 75% and 71.7% at salinity levels 0.25%, 0.50% and 0.75% respectively whereas it remained static to 93.3% under control condition. Thus, salinity had little inhibitory effect on the germination.

Shoot length: The average shoot length of the susceptible type of seedlings gradually decreased with increasing salinity level. The shoot length was 2.25 cm, 1.74 and 1.52 cm at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of seedlings growing under control the average shoot length was 3.08 cm, which reduced to 2.40, 2.11 and 1.36 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the susceptible seedlings was 1.52, 0.91 and 0.68 cm at 0.25%, 0.50% and 0.75% salinity levels respectively. In the resistant group of seedlings growing under control the average root length of the seedlings was 2.97 cm, which reduced to 1.63, 1.56 and 0.72 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the susceptible type of plants was 1.11, 0.86 and 0.86 at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of seedlings growing under control condition the average number of leaves were 2.35, which reduced to 1.07, 0.98 and 0.98 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plants: The average biomass production of the susceptible type of seedlings was very marginally affected under salt-stress conditions. The biomass production was 32 mg, 32 mg and 29 mg at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of seedlings growing under control condition the average biomass production was 34mg, which was almost static at all, the salinity treatments. The biomass production was 31mg, 31 mg and 28 mg at 0.25%, 0.50% and 0.75% salinity respectively.

Penta 99

Germination: Germination at 0.25% and 0.50% was almost equal to the control condition whereas it reduced at 0.75%. By 10th day the germination was 55%, 23.3% and 3.3% at 0.25%, 0.50% and .075% respectively whereas under control condition it was 61.6%. By 20th day the germination was 63.3%, 65% and 31.7% at 0.25%, 0.50% and 0.75% salinity whereas under control condition it was 70%.

Shoot length: The average shoot length of the susceptible type of seedlings was 1.67, 1.71 and 1.25 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant type of seedlings growing under control the average shoot length of seedlings was 3.21 cm, which increased to 4.10 at 0.25% salinity whereas at 0.50% and 0.75% it was 2.40 and 1.29 cm.

Root length: The average root length of the susceptible type of seedlings was significantly reduced to 0.60, 0.72 and 0.79 cm at 0.25%, 0.50% and 0.75% salinity levels respectively. The roots had abnormal growth pattern i.e. the roots were aerial, hair-like and negatively geotropic. In the resistant type of seedlings growing under control condition the average root length was 2.32 cm, which was marginally reduced to 1.74, 1.29 cm at 0.25%, 0.50% salinity level respectively but at 0.75% it was very significantly reduced to 0.74 cm.

Number of leaves: The average number of leaves in the susceptible type of seedlings was 1.41, 0.84 and 1.20 at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant type of seedlings growing under control condition the average number of leaves were 2.22, which were reduced to 1.75, 1.01 and 1.38 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plant: The average biomass production of the susceptible type of seedlings was marginally reduced to 44 mg, 44 mg and 50 mg at 0.25%, 0.50% and 0.75% salinity levels respectively. In the resistant type of seedlings growing under control condition the average biomass production was 56 mg, which was almost static at all, salinity treatments. It was 50 mg, 53 mg and 50 mg at 0.25%, 0.50% and 0.75% salinity levels respectively. Thus increasing salinity levels had little affect as far as biomass production was concerned.

Raj Bundi

Germination: The overall germination was reduced under salt stress condition as compared to control. During the initial period of exposure to salinity i.e. by 10th day it was 48.3%, 20% and 13.3% at 0.25%, 0.50% and 0.75% salinity level whereas under

control condition it was 71.7%. During the later period i.e. by 20th day the germination was 58.3%, 45% and 35% at 0.25%, 0.50% and 0.75% salinity level whereas under control condition it remained static to 71.7% germination.

Shoot length: The average shoot length of the susceptible type of seedlings was 2.47, 1.98 and 1.71 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant group of seedlings growing under control the average shoot length of the seedlings was 4.17 cm, which was reduced to 3.54, 1.77 and 1.73 at 0.25%, 0.50% and 0.75% salinity level respectively.

Root length: The average root length of the susceptible type of seedlings was highly reduced to 0.89, 0.79 and 0.84 at 0.25%, 0.50% and 0.75% salinity levels respectively. The roots that had emerged had abnormal pattern of growth i.e. the roots were aerial, hair-like and negatively geotropic. In the resistant type of seedlings the average root length was marginally reduced at 0.25% and 0.50% to 2.33 cm and 1.83 cm respectively and 0.84 cm at 0.75% as compared to 2.69 cm in control.

Number of leaves: The average number of leaves in the susceptible type of seedlings was marginally reduced to 1.54, 1.57 and 1.09 at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant group the leaves per seedling was 2.02, 1.01 and 1.28 at 0.25%, 0.50% and 0.75% salinity respectively as compared to 2.32 in control.

Weight of plant: The average biomass production of the susceptible type of seedlings was 74mg, 106 mg and 68 mg at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant group of seedlings the biomass of the seedlings was 50 mg, 50 mg and 56 mg at 0.25%, 0.50% and 0.75% salinity levels respectively compared to 59 mg in control.

Penta 99-1

Germination: Germination showed declining trend with increasing salinity level. Percent germination was 61.7%, 46.7% and 45% at 0.25%, 0.50% and 0.75% respectively whereas it was 72% under control condition. Germination under salt stress initiated after the 5th day in the treatments whereas under control condition it initiated on 3rd day. Delayed germination was also evident with 35.6%, 21.6% and 16.6% under 0.25%, 0.50% and 0.75% salinity compared to 58.3% under control conditions by 10th day.

Shoot length: The average shoot length of the susceptible type of seedlings was marginally reduced to 2.33, 2.43 and 1.57 cm at 0.25%, 0.50% and 0.75% salinity level respectively. In the resistant type of seedlings also the shoot length was marginally reduced at 0.25% to 2.71, at 0.50% and 0.75% salinity, it was reduced to 1.67 and 1.62 cm respectively compared to 3.25 cm under control.

Root length: The average root length of the susceptible seedlings was 1.94, 1.02 and 0.78 at 0.25%, 0.50% and 0.75% salinity respectively. In seedlings growing under control condition the average root length was 2.73 cm, which was marginally reduced among the tolerant seedlings at 0.25% and 0.50% salinity; it was 2.10 cm and 2.57 cm respectively. However at 0.75% salinity the root length was substantially reduced to 0.71 cm.

Number of leaves: The average number of leaves in the susceptible plants was 1.58, 1.68 and 1.12 at 0.25%, 0.50% and 0.75% salinity level respectively. The resistant type of plant the number of leaves was 2.02, 1.11 and 1.52 at 0.25%, 0.50% and 0.75% salinity respectively compared to that growing under control condition wherein the average leaf number was 2.47,

Weight of plant: The average biomass production in the susceptible seedlings was 121 mg, 121 mg and 129 mg at 0.25%, 0.50% and 0.75% salinity respectively. In the resistant type of plants growing under control condition the average biomass production was 62 mg which was unaffected at 0.25% salinity and remained stable at 62 mg but at 0.50% and 0.75% salinity the biomass production was reduced to 50 mg and 56 mg respectively.

ES 99

Germination: The rate of germination showed a declining trend with increasing salinity levels. Percent germination was observed to be 66.7%, 43.3% and 21.7% at 0.25%, 0.50%, and 0.75% salinity levels, whereas it was 83.3% under control conditions. Even the process of germination was delayed at higher salinity levels. As most of the seeds under control condition germinated by 10th day, whereas only 33.3% at 0.50% and 1.6% of the seeds germinated at 0.75% salinity.

Shoot length: The length of shoot decreased with increasing salinity levels. Susceptible plants at 0.25% salinity level had 2.87 cm average length of shoot, which decreased marginally to 2.58 cm at 0.50% salinity and at 0.75% salinity it further decreased to 1.64 cm. Resistant type of plants at 0.25% salinity level had an average height of 4.02 cm, which decreased marginally at 0.50% salinity to 3.08 cm but the reduction in growth was substantial at 0.75% salinity where it reduced to 1.77 cm whereas under control conditions the average height of plants was 5.50 cm.

Root length: Susceptible types of plants were most affected as for as the development of roots was concerned. The average root length at 0.25% salinity was 1.20 cm, at 0.50% it was 1.68 cm and at 0.75% salinity it reduced to 0.82 cm. Resistant types of plants also showed a decreasing trend with increasing salinity but the reduction was marginal at 0.25% and 0.50%. The average length of roots at 0.25% salinity was 3.82 cm, whereas at

0.50% salinity it was 3.02 cm and at 0.75% salinity the root development was drastically reduced to 0.82 cm, whereas under control condition the average length of root was recorded to be 4.26 cm.

Number of leaves: The number of leaves at 0.25%, 0.50% and 0.75% salinity was 1.79, 1.61 and 1.61 respectively in the susceptible type of plants. In the resistant type of plants 2.35, 1.77 and 1.77 leaves were present at 0.25%, 0.50% and 0.75% salinity level, whereas 3.53 leaves were present in the plants growing under control. Thus the development of leaves was affected due to salinity, though the number of leaves at 0.75% and 0.50% salinity was almost similar and highly reduced compared to control.

Weight of plants: The susceptible type of plants in this genotype had increased biomass as compared to resistant type of plant due to succulent nature of leaves. The average weight of plants at 0.25% salinity was 80 mg; at 0.50% salinity it was 94 mg, whereas at 0.75% salinity the weight of seedling was 40 gm. The biomass of seedlings of resistant type of plants was 68mg, 63 mg and 44 mg at 0.25%, 0.50% and 0.75% salinity level respectively whereas under control condition it was 107 mg. Thus a gradual decline in biomass of seedlings was observed with increasing salinity levels.

ISH 32/8/1

Germination: Among all the salinity levels the process of germination initiated after 5 days and only 1.16%, 8.3 and 0% germination could be observed by 10th day at 0.25%, 0.50% and 0.75% salinity level respectively, whereas 83.3% of the seeds germinated in control. By 20th day 48.3%, 31.7%, and 20% of the seeds germinated at 0.25%, 0.50% and 0.75% salinity levels whereas 85% seeds germinated under control.

Shoot length: The growth of shoot had a decreasing trend with increasing salinity level. Susceptible plants at 0.25% salinity level had average height of 2.40 cm, which decreased to 1.93 cm at 0.50% salinity level and to 1.43 cm at 0.75% salinity level compared to 3.15 cm in control. The resistant type of plants had average length of 2.51 cm at 0.25% salinity, 1.99 cm at 0.50% and 1.49 cm at 0.75% salinity. Overall growth response of the shoot portion at varying level of salinity indicated decreasing trend. The reduction in growth was prominent in 0.50% and 0.75%.

Root length: The average length of root at 0.25% salinity was 1.38 cm, which decreased to 1.18 cm at 0.50% salinity. The reduction in root length was more pronounced at 0.75% where the average length of root was 0.60 cm, whereas the average length of roots in control was 3.18 cm. Thus the inhibitory affect of salt treatment on the growth of root was more evident at higher salinity level. The roots in the susceptible type of plants were

negatively geotropic, aerial, hair like. The resistant type of plants at 0.25% had an average length of 1.39 cm, at 0.50% the length was 1.17 cm and at 0.75% the length was 0.52 cm whereas the plants growing under control had 3.46 cm long roots. Thus, average length of root at 0.25% was reduced to half as compared to control. The reduction was more at higher salinity level i.e. 0.50% and 0.75%.

Number of leaves: The growth and development of leaves was marginally affected due to salinity. In the susceptible type of plants at 0.25% salinity the average number of leaves was 1.61, at 0.50% and 0.75% the average number of leaves was 1.61 whereas in control condition the average leaves were 1.79. Among resistant plants the average number of leaves at 0.25% salinity was 1.94 and at 0.50% and 0.75% the average number of leaves was 1.77. The average number of leaves under control conditions was 1.96, thus a marginal reduction in number of leaves was observed at salinity level 0.50% and 0.75%, whereas the average leaf count at 0.25% and control was almost equal. Thus, the average count of leaves at varying salinity levels as compared to the control plants showed reduction.

Weight of Plant: The biomass of plants in general showed a declining trend with increasing salinity level. The susceptible type of plant at 0.25% salinity had an average biomass of 70 mg, at 0.50% it was 55 mg and at 0.75% it was 35 mg, whereas the average weight of seedlings under control condition was 70 mg. In the resistant type of plants growing at 0.25% salinity the average biomass of the seedlings was 68 mg, at 0.50% biomass was 54 mg and at 0.75% the biomass was 34 mg whereas the average biomass of seedlings growing in control was 73 mg.

Wardan S2

Germination: Ten days after inoculation the germination percent observed was 43.3%, 21.6% and 5% at 25%, 0.50% and 0.75% salinity level respectively which improved to 61.7%, 38.3%, and 28.3% by 20th day. However, under control condition, by 20th day 86.7% germination was observed. Thus, increasing salinity levels had adverse effect on germination.

Shoot length: Among the susceptible type of plants growing *in vitro* at 0.25% salinity the average shoot length was 1.71 cm, which was 1.72 cm at 0.50% salinity and 0.71 cm at 0.75% salinity. In the resistant plants shoot length was 1.89, 2.27 and 0.73 cm at 0.25%, 0.50% and 0.75% salinity respectively whereas the average shoot length of seedlings in control condition was 4.47 cm. Thus, reduction in shoot length was observed at 0.75% with marginal increase at 0.50% salinity as compared to control.

Root length: The growth and development of roots was inhibited under all salt treatments. In the susceptible type of seedlings the growth of roots was inhibited and followed abnormal pattern of growth with aerial/hair like roots. At 0.25% salinity the average length of root was 0.60 cm, at 0.50% salinity it was 0.40 cm and 0.75% salinity it was observed to be 0.37 cm whereas under control condition root length was 2.59 cm. Root growth among some of the plants was marginally better. Thus, in the other group of plants at 0.25% salinity the average length of root was 0.65 cm, at 0.50% length was 1.52 cm and at 0.75% salinity length was 0.39 cm.

Number of leaves: Leaf initiation was substantially reduced under stress condition as compared to control. In the susceptible type of plants at 0.25% salinity the average number of leaves was 1.30, at 0.50% it was 1.12 and at 0.75% salinity it was 0.84, whereas under control conditions it was 2.24. Thus, negative impact was more pronounced at 0.75% salinity level. Among resistant type of plants leaf formation was somewhat better and at 0.25% salinity 1.36 leaves, at 0.50% salinity 1.76 leaves and at 0.75% salinity 0.97 leaves were observed as compared to 2.65 leaves in control condition. However, the average number of leaves increased at 0.50% due presence of a few plants which possessed more leaves.

Weight of Plant: The average biomass production in the genotype was reduced when exposed to saline conditions. In the susceptible type of plants at 0.25% salinity level the average weight of seedlings was 35 mg, at 0.50% salinity level it was 42 gm and at 0.75% it was 32 mg. The resistant type of plants at 0.25% salinity level had average biomass production of 41 mg, at 0.50% the average weight of seedlings was 59 mg and at 0.75% salinity it was 32 mg. However, the average biomass production under control condition was 66 mg. Thus, most of the seedling showed almost same response to salinity.

ISH 26/50/7

Germination: Germination was reduced as compared to control. By 10th only 13.3% of the seeds germinated at 0.25% salinity and by 20th day 41.7% germination was also observed. Germination at 0.50% initiated after the 5th day and by 10th day only 6.6% of the seeds germinated which improved to 20.0% by 20th day. At 0.75% germination was almost inhibited and by 20th day only 13.3% of the seeds germinated. In control condition, by 10th day 46.6% seeds germinated and by 20th day 91.7% seeds germinated.

Shoot length: The average shoot length in both the susceptible and resistant type of plants decreased with increasing salinity level. In the susceptible type of plants at 0.25% salinity the average length of shoot was 1.04 cm, at 0.50% salinity the average length of

shoot was 1.07 cm and at 0.75% salinity it reduced to 0.45 cm. Thus the average shoot length at 0.25% and 0.50% salinity was almost equal though reduced but at 0.75% salinity the development of shoot was almost inhibited. In the resistant type of plants at 0.25% salinity the average shoot length was 3.08cm, at 0.50% salinity it was 1.11 cm and at 0.75% the average shoot length was reduced to 0.45cm, whereas the shoot length in control conditions was 4.21 cm. Thus, the increasing salinity level reduced the development of shoot and at 0.75% salinity level the development of shoot was almost inhibited.

Root length: In the susceptible type of plants at 0.25% salinity the average root length was 0.46 cm, at 0.50% salinity it was 0.58 cm and at 0.75% salinity it was 0.27cm. Thus, the development of root of the seedlings growing at different salinity levels was almost inhibited and whatever growth was observed was abnormal with aerial, hair like roots. In the resistant type of seedlings at 0.25% salinity the average root length was 1.81 cm, at 0.50% salinity it was 0.54 cm and at 0.75% it was 0.33 cm, whereas the average root length in control was 3.58 cm. Thus, salinity above 0.25% had an inhibitory effect on the development of root.

Number of leaves: The growth and development of leaves was substantially reduced with increasing salinity levels. In the susceptible type of plants at 0.25% salinity the average number of leaves was 1.26, at 0.50% it was 1.21 and at 0.75% it was 0.92. In the resistant type of plants at 0.25% salinity the average number of leaves was 1.87 cm, at 0.50% it was 0.97 and at 0.75% it was 0.88, whereas the average number of leaves in control was 2.95. Thus, salinity above 0.25% had an inhibitory effect on leaf initiation.

Weight of plants: The average biomass production at different salinity level was significantly reduced. The susceptible type of plants at 0.25% salinity had an average weight of 32 mg, at 0.50% it was 42 mg and at 0.75% salinity it was 17 mg. In the resistant type of plants at 0.25% salinity the average seedlings weight was 41 mg, at 0.50% it was 41 mg and at 0.75% it was 17 mg, whereas the biomass per plant under control was 68 mg. The result indicates that the biomass production was substantially reduced with increasing salinity levels.

ISH 32/34/1

Germination: Germination during the initial period of exposure to saline conditions i.e. by 10th day was 0%, 3.3%, 0% and 26.6% at 0.25%, 0.50%, and 0.75% and under control conditions. By 20th day it improved to 63.3%, 18.3%, 8.7% and 76.7% at 0.25%, 0.50%,

and 0.75% and control respectively. Thus germination was almost inhibited at 0.75% salinity.

Shoot length: In the susceptible type of plants at 0.25% salinity the average shoot length was 1.05 cm, at 0.50% at was 1.01 cm and at 0.75% salinity it was 0.37 cm. In the resistant type of plants the average shoot length at 0.25% salinity was 3.05cm; at 0.50% salinity level it was 2.80 cm and at 0.75% it was 0.38 cm, whereas the average shoot length of the seedlings under control was 3.71cm. Thus, growth of the seedlings was marginally affected at 0.25% and 0.50% salinity level and almost inhibited at 0.75%.

Root length: In the susceptible type of plants at 0.25% salinity the average root length was 0.64 cm, at 0.50% it was 0.39 cm and at 0.75% it was 0.39cm. The root exhibited abnormal growth i.e. the roots were aerial/hair like. In the resistant type of plants at 0.25% salinity the average root length was 1.52 cm, at 0.50% it was slightly increased to 2.09 cm and at 0.75% it was reduced to 0.33 cm, whereas in the seedlings growing under control condition the average root length was 2.51 cm. Thus, the reduction was marginal at 0.25% and 0.50% salinity and almost inhibited at 0.75%.

Number of leaves: In the susceptible type of plants at 0.25% salinity the average number of leaves was 1.26, at 0.50% it was 0.93 and at 0.75% it was again 0.93. In the resistant type of plants at 0.25% salinity the average number of leaves was 1.57, at 0.50% it was slightly increased to 1.95 and at 0.75% it was again reduced to 0.88, whereas the average number of leaves under control conditions was 2.36.

Weight of plant: In the susceptible type of plants at 0.25% salinity the average biomass production was 37 mg, at 0.50% salinity it was 27 mg, and at 0.75% salinity it was 17 mg. In the resistant type of plant at 0.25% salinity the average weight of seedlings was 59 mg, at 0.50% it was increased to 73 mg and at 0.75% it was reduced substantially to 17 mg, whereas under control conditions it was 61 mg.

Multi-98-45

Germination: This genotype showed reduced and delayed germination with increasing salinity level. By 10th day 3.3%, 0%, 0% seeds germinated under 0.25%, 0.50% and 0.75% salinity whereas in control 23.3% seeds germinated. The rate of germination during the later phase i.e. by 20th day was 48.3%, 16.7%, 11.7% and 80% under 0.25%, 0.50%, 0.75% salinity and control conditions respectively.

Shoot length: The growth and development of shoot was reduced substantially with increasing salinity level. In the susceptible type of plants at 0.25% salinity the average shoot length was 1.62 cm, at 0.50% it was reduced to 0.72 cm and at 0.75% salinity it was

0.64 cm, whereas the average shoot length in control was 3.68 cm. Thus, the reduction was more pronounced at higher salinity level. Some plants, which exhibited tolerant nature, attained 1.70 cm height at 0.25% salinity, 0.74 cm at 0.50% and 0.55 cm at 0.75%.

Root length: In the susceptible type of seedlings the development of root was highly inhibited and had abnormal growth with aerial/hair like roots. The average length of root at 0.25% salinity was 0.85cm, at 0.50% it was 0.56 cm and at 0.75% it was 0.30 cm, whereas the average root length under control condition was 3.0 cm. In the resistant type of seedlings at 0.25% salinity level the average root length was 1.45 cm, at 0.50% it was 0.57 cm and at 0.75% it was 0.26 cm. Thus, the development of roots under salt stress was inhibited, although at 0.25% salinity some of the plants showed satisfactory development of roots.

Number of leaves: The growth and development of leaves was highly inhibited under salt stress. In the susceptible type of plants the average number of leaves at 0.25% was 1.12, at 0.50% it was 0.97 and at 0.75% it was 0.88, whereas under control condition it was 2.34. The tolerant seedlings at 0.25% salinity developed 0.81 leaves per plant as compared to 0.81 leaves per plant at 0.50% and 0.75% salinity.

Weight of Plant: The biomass production was substantially reduced under salinity as compared to control. In the susceptible type of plants the average weight of seedlings at 0.25% salinity was 48 mg, at 0.50% it was 22 mg and at 0.75% it was 20 mg, whereas under control condition it was 71 mg. In the resistant type of plants at 0.25% salinity, the average biomass of the seedlings was 49 mg, at 0.50% it was 19 mg and at 0.75% it was 14 mg. Thus, the biomass production was reduced proportionally to increasing salinity level.

ISH 34/5/1

Germination: Germination was inhibited as the level of salinity was increased. Even the process of germination was delayed due to salinity. During the initial exposure to saline conditions i.e. by 10th day 13.3%, 0%, 0% and 53.3% germination was reported at 0.25%, 0.50%, 0.75% and control condition. During the later phase i.e. by 20th day the germination rate was 65%, 33.3%, 11.7% and 76.7% at 0.25%, 0.50%, 0.75% and control conditions respectively.

Shoot length: Growth and development of shoot was inhibited under salt stress. In susceptible group of plants at 0.25% salinity the average shoot length was 1.14 cm, at 0.50% it was 0.93 cm and at 0.75% it was reduced to 0.49 cm. Among the plants which showed some tolerance at 0.25% salinity the average shoot length was 1.96 cm, at 0.50%

it was 1.24 cm and at 0.75% it was reduced significantly to 0.41 cm, whereas under control condition the average shoot length was 3.29 cm. Thus, the reduction was more significant at 0.75% salinity.

Root length: The growth and development of root was inhibited to some extent in the resistant type of plants and almost completely inhibited in susceptible type of plants. In the susceptible type of plants at 0.25% salinity the average root length was 0.69 cm, at 0.50% it was 0.43 cm and at 0.75% salinity it was 0.21 cm. Many had abnormal growth having aerial/ hair like roots. In the resistant plants at 0.25% salinity the average root length was 1.48 cm, at 0.50% it was 1.40 cm and at 0.75% it was 0.31 cm, whereas under control condition the average root length was 2.37 cm. Thus, salinity did have inhibitory effect on the growth of roots but this effect was more pronounced at 0.75% salinity.

Number of leaves: The growth and development of leaves was reduced at increasing salinity level. In the susceptible type of plants at 0.25% salinity the average number of leaves was 1.26, at 0.50% it was 1.20 and at 0.75% it was 0.97. In the resistant type of plants at 0.25% the average number of leaves was 1.17, at 0.50% it was 0.90 and at 0.75% it was 0.81, as compared to 2.33 in control. Though, the number of leaves decreased with increasing salinity level in both the resistant and susceptible type of plants, yet the reduction was more pronounced in the resistant type of plant.

Weight of plant: The biomass production was increased at 0.25% salinity in the susceptible type of plants whereas in the resistant type of plants at the same salinity it was almost equal to that under control condition but with the increasing level of salinity the biomass decreased. In the susceptible type of plants at 0.25% salinity the average weight of seedlings was 59mg, at 0.50% it was 42 mg and at 0.75% it was 22 mg. In the resistant type of seedlings at 0.25% salinity level the average weight of seedlings was 44 mg, at 0.50% it was increased marginally to 46 mg and at 0.75% it was reduced substantially to 19 mg, whereas under control condition it was 55 mg. Thus, an interesting feature of increase in the biomass production at low salinity level in the susceptible type of plants as compared to control and at higher salinity level i.e. 0.50% as compared to 0.25% salinity was observed.

Raj 49/50

Germination: The rate of germination was highly reduced, delayed and completely inhibited above 0.25% salinity. At 0.25% salinity only 10% of the seeds germinated as compared to 45% germination in control condition. Thus, salinity had a highly deleterious effect on the process of germination in this genotype.

Shoot length: The length of shoot was substantially reduced under 0.25% salinity as compared to control in both the susceptible and resistant type of plants. The average shoot length at 0.25% salinity in the susceptible type of plants was 0.75cm. In the resistant type of seedlings at 0.25% salinity the average shoot length was 0.62cm whereas under control condition the average shoot length was 2.71cm. Thus salinity had an inhibitory effect on the growth and development of shoot.

Root length: The growth and development of root was highly inhibited in both the susceptible and resistant type of seedlings at 0.25% salinity as compared to control. Some of the seedlings showed the growth of root in upward direction with some hair like roots. Data recorded on two groups of the plants showed 0.66cm and 0.59 cm long roots, whereas under control condition it was up to 2.35cm.

Number of leaves: The growth and development of leaves was highly inhibited under salt stress as compared to control conditions. Almost all the seeds responded in same way with regard to leaf initiation. Data recorded in two groups showed little variation. In the susceptible group the average number of leaves was 0.88 whereas in the other group the number of leaves was 0.81. Among control plants the average number of leaves was 1.63.

Weight of plant: The biomass production under salt stress was substantially reduced. On an average the weight of seedlings was 22 mg at 0.25% salinity, whereas under control conditions it was 46 mg.

T 44 – 4

Germination: Germination was reduced and delayed at varying levels of salinity as compared to control. By 10th day no germination was observed under all salinity levels, whereas in control 40% of the seed germinated. By 20^{th} day 51.7%, 16.7%, 16.7% and 85% germination was observed at 0.25%, 0.50%, 0.75% and control condition.

Shoot length: In the susceptible group of plants at 0.25% salinity the average shoot length was 1.28 cm, at 0.50% it was decreased to 1.00 cm and at 0.75% it was 1.08 cm. Thus a gradual decrease in shoot length with increasing salinity level was observed. In the resistant type of plants the average shoot length at 0.25% salinity level was 2.73 cm, at 0.50% salinity it was reduced substantially to 0.82 cm and at 0.75% it was 0.97 cm, whereas under control condition it was 4.76 cm. Thus shoot length was substantially reduced under saline condition as compared to control but this reduction was more manifested at 0.50% and 0.75% salinity level though a marginal increase was observed at 0.75% as compared to 0.50% salinity level.

Root length: In the susceptible type of plants at 0.25% the average length of root was 1.80 cm, at 0.50% it was drastically reduced to 0.50 cm and at 0.75% it was 0.74 cm. Thus, the development of root was highly retarded under saline conditions and the roots had abnormal pattern of growth with aerial/hair like roots. In the resistant type of plants at 0.25% the average root length was 1.68 cm, at 0.50% it was drastically reduced to 0.39 cm and at 0.75% it marginally increased to 0.54 cm, whereas under control condition the average root length was 8.03 cm. Thus, a significant decrease in the average root length was observed under saline condition as compared to control.

Number of leaves: In the susceptible type of plants at 0.25% it was 0.75, at 0.50% and 0.75% it was 0.75. In the resistant group of plants at 0.25% the average number of leaves was 1.51, at 0.50% it reduced to 0.76 and at 0.75% it marginally increased to 0.84, whereas under control condition the average number of leaves was 2.43. Thus, salinity had an inhibitory effect on the development of leaves in the growing seedlings and this was more sever at 0.50% and 0.75% salinity level.

Weight of plant: In the susceptible type of seedlings at 0.25% the average biomass production was 58 mg, at 0.50% it marginally reduced to 48 mg and at 0.75% it substantially reduced to 27 mg. In the resistant type of plants at 0.25% the average biomass of the seedlings was 87 mg, at 0.50% it was greatly reduced to 37 mg and at 0.75% it was 28 mg only, whereas under control condition the average biomass production was 152 mg. Thus, the average biomass production under saline conditions was greatly reduced as compared to control.

T 45-1

Germination: During the initial period of exposure to saline conditions i.e. by 10th day germination was 6.6%, 1.6%, 10% and 85% under 0.25%, 0.50%, 0.75% and control conditions. By the 20th day the germination was 53.3%, 16.7%, 15% and 100% at 0.25%, 0.50%, 0.75% and control condition respectively.

Shoot length: The growth of shoot under salt stress condition was substantially reduced as compared to control. In the susceptible group of plants at 0.25% salinity the average shoot length was 1.72cm, at 0.50% it was 1.14 cm and at 0.75% it was 1.16 cm. In the resistant type of plants at 0.25% salinity the average shoot length was 2.57 cm, at 0.50% it was 2.31 cm and at 0.75% it was reduced to 0.97cm, whereas under control it was 5.10 cm. Thus, the reduction in shoot length was more significant among some susceptible type of plants under saline condition, whereas in the resistant type of plants the growth of shoot was significantly reduced at higher salinity level i.e. at 0.75% salinity.

Root length: In the susceptible type of plants at 0.25% salinity the average root length was 1.01 cm, at 0.50% it was reduced significantly to 0.19cm and at 0.75% it increased to 0.73cm. In the resistant type of plant at 0.25% salinity the average root length was 2.30 cm, at 0.50% it was drastically reduced to 0.94 cm and at 0.75% it was 0.60 cm, whereas under control condition the average root length was 4.52 cm. Thus the growth and development of roots was inhibited with increasing salinity level. The susceptible type of plants had retarded and abnormal growth of roots with aerial, hair-like roots. The development of roots was almost inhibited at salinity 0.50% onward.

Number of leaves: In the susceptible type of plants the average number leaves at 0.25% salinity was 1.49, at 0.50% it increased to 2.15 and at 0.75% it was reduced to 0.83. In the resistant type of seedlings the average number of leaves was 1.51, at 0.50% it was 1.34 and at 0.75% it was 0.76, whereas under control condition it was 2.35. Thus the growth of leaves was inhibited with increasing salinity level and at 0.75% it was very much retarded.

Weight of plant: The average biomass production constantly decreased with increasing salinity level. In the susceptible type of plants at 0.25% salinity the average biomass was 95 mg, at 0.50% it was 58 mg and at 0.75% it was 41 mg, whereas under control condition the average biomass production was 78mg. Thus the average biomass production under 0.25% salinity was increased as compared to control and other salinity treatments. In the resistant type of seedlings at 0.25% salinity the average weight of the seedlings was 62 mg, at 0.50% it was 53 mg and at 0.75% it was reduced to 31 mg. Thus, a gradual decrease in the biomass production with increasing salinity level was observed.

T 5-90I-1

Germination: The germination percent was reduced and delayed due to salinity. By 10th day the was 0%, 0%, and 2.5% seeds germinated at 0.25%, 0.50% and 0.75% salinity as compared to 31.6% in control. By 20th day germination improved to 46.7%, 10% and 10% respectively in 0.25%, 0.50% and 0.75% salinity. Germination in control was 88.3%. Thus, the germination was highly affected at 0.50% and 0.75% salinity level.

Shoot length: The growth and development of shoot was reduced under salt stress conditions as compared to control. In the susceptible type of plants at 0.25% salinity the average shoot length was 1.41 cm, at 0.50% it reduced to 0.29 cm and at 0.75% it increased to 1.39 cm, whereas under control condition the average shoot length was 3.23 cm. Some plants showing tolerant behaviour attained an average shoot length of 1.38 cm at 0.25% salinity, 0.27 cm at 0.50% and 1.25 cm at 0.75%.

Root length: Growth and development of root under salt stress was significantly reduced as compared to control. In the susceptible type of plants at 0.25% salinity the average shoot length was 0.36 cm, at 0.50% it was 0.19 cm and at 0.75% it was 0.36 cm. In the resistant type of plants at 0.25% salinity the average root length was 2.30 cm, at 0.50% it was 0.94 cm and at 0.75% it was 0.26 cm, whereas under control condition it was 3.13 cm. Thus the average root length decreased with increasing salinity level. In some of the plants growth of roots was almost inhibited and the roots were aerial/hair – like. In the resistant type of plants the growth of root at 0.25% was good but as the salinity was increased, reduction in root growth was observed.

Number of leaves: The growth and development of leaves was also inhibited under salt stress condition. In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 1.08, at 0.50% it was 0.75 and at 0.75% it was 0.75, whereas under control condition the average number of leaves was 2.06. Among tolerant group of plants at 0.25% salinity the average number of leaves was 0.92, at 0.50% it was 0.76 and at 0.75% it was 0.76.

Weight of plant: The biomass production in the susceptible type of plants at 0.25% salinity was 58 mg, at 0.50% it was 17 mg and at 0.75% it was increased slightly to 34 mg, whereas the control plants weighed 71 mg. Among the resistant type of seedlings at 0.25% salinity the average weight of seedlings was 90 mg, at 0.50% it was significantly reduced to 12 mg and at 0.75% it was observed to be 22 mg. Thus, marginal increase in plant weight noticed at 0.25% salinity followed significant reduction in biomass production at higher salinity level.

ISH 8020B

Germination: By 10^{th} day germination was 33.3%, 13.3%, 8.3% and 98.3% at 0.25%, 0.50%, 0.75% and control condition respectively which improved to 68.3%, 46.7%, 25%, and 98.3% by 20^{th} at 0.25%, 0.50%, 0.75% and control condition respectively.

Shoot length: In the susceptible type of plant at 0.25% salinity the average shoot length was 0.81 cm, at 0.50% it marginally increased to 1.59 cm and at 0.75% it reduced to 0.98 cm. In the resistant type of plant at 0.25% the average shoot length of the seedlings was 3.67 cm, at 0.50% it was 2.31 cm, at 0.75% salinity it was 1.71 cm whereas, under control condition the average shoot length of the seedlings was 5.98 cm. Thus the average shoot length under increasing salinity level decreased constantly.

Root length: In the susceptible type of plants at 0.25% salinity the average root length was 1.23 cm, at 0.50% it was reduced to 0.53 cm and at 0.75% the growth of roots was

almost inhibited to 0.38 cm. In the resistant type of plant at 0.25% the average root length was 4.40 cm, at 0.50% it reduced to 1.67 cm and at 0.75% it further reduced to 0.55 cm, whereas under control the average root length of the seedlings was 5.51 cm.

Number of leaves: In the susceptible type of plant at 0.25% salinity the average number of leaves was 0.91, at 0.50% it was 0.74 and at 0.75% it was marginally more as compared to 0.25% and 0.50% it was 0.94. In the resistant type of plants at 0.25% salinity the average number of leaves was 1.86, at 0.50% it was marginally reduced to 1.52 and at 0.75% it was 1.35, whereas under control condition the average number of leaves was 2.76. Thus growth and development of leaves gradually declined with increasing salinity level.

Weight of plant: In the susceptible type of plants at 0.25% salinity the average biomass production was 42 mg, at 0.50% it was marginally increased to 56 mg and at 0.75% it was reduced to 39 mg. In the resistant type of plants at 0.25% the biomass production was 87 mg, at 0.50% it was reduced to 60 mg and at 0.75% was further reduced to 42 mg, whereas under control condition the average biomass production was 144 mg. thus a gradual decline in the average biomass production with increasing salinity level was observed.

ISH 8020 Y

Germination: The overall germination under saline conditions was reduced as compared to control conditions. Under control conditions 91.7% germination was observed by the 10^{th} day, whereas 30%, 5% and no germination was observed at 0.25%, 0.50% and 0.75% salinity respectively. The final germination observed on the 20^{th} day was 56.7%, 30%, 18.3% and 91.7% in 0.25%, 0.50%, 0.75% and control conditions respectively. Thus with increasing salinity level the rate of germination was reduced.

Shoot length: In the susceptible type of seedlings at 0.25% the average shoot length was 2.20 cm, at 0.50% it was 1.11 cm and at 0.75% it was 1.02 cm. In the resistant type of seedlings at 0.25% the average shoot length was 2.79 cm, at 0.50% it was 1.98 cm and at 0.75% it was greatly reduced to 0.83cm, whereas under control condition the average shoot length of the seedlings was 6.25cm. Thus salinity inhibited the growth and development of shoot and this inhibitory effect was more severe at higher level of salinity i.e. at 0.50% and 0.75%.

Root length: In the susceptible type of plants at 0.25% salinity the average root length of the seedlings was 0.35cm, at 0.50% it was 0.23cm and at 0.75% it was 0.35cm. This indicates that saline conditions are highly inhibitory to the development of root in this

genotype; in fact the roots that were present had abnormal growth i.e. the roots were aerial and hair like.

In the resistant type of seedlings at 0.25% the average root length was 1.98 cm, at 0.50% it was reduced to 0.87 cm and at 0.75% it was inhibited to just 0.26cm, whereas under control condition the average root length was 4.95cm.

No. of leaves: In the susceptible type of plants at 0.25% salinity the average number of leaves was 2.47, at 0.50% it was substantially reduced to 0.82 and at 0.75% it was again 0.82. Thus the average number of leaves at 0.25% was more than at control and the other two higher saline treatments. In the resistant type of seedlings at 0.25% the average number of leaves was 1.73, at 0.50% it was reduced to 1.11 and at 0.75% it was further reduced to 0.62, whereas under control condition the average number of leaves was 2.07. Thus the growth and development of leaves gradually decreased with increasing salinity level.

Weight of plant: The biomass production declined as the level of salinity increased in both the susceptible and resistant type of plants. In the susceptible type of seedlings at 0.25% salinity the average biomass production was 70 mg, at 0.50% it was decreased to 37 mg and at 0.75% it showed no change and remained 37 mg. In the resistant type of seedlings the average weight of seedlings at 0.25% salinity was 58 mg, at 0.50% it was 42 mg and at 0.75% it was 32 mg, whereas under control conditions the average biomass production of the seedlings was 100 mg. Thus the biomass production of the seedlings was substantially reduced under saline conditions as compared to control.

ISH 5050 B

Germination: The rate of germination was substantially reduced with increasing salinity level both during the initial period of exposure to saline conditions i.e. by 10th day and late period i.e. by the 20th day. The rate of germination on the 10th day was 51.6%, 11.6%, 1.16% and 86.6% at 0.25%, 0.50%, 0.75% and control condition whereas by the 20th day it 80%, 33.3%, 38.3 and 93.3% at 0.25%, 0.50%, 0.75% and control conditions respectively.

Shoot length: The growth and development of shoot was reduced with increasing salinity level as compared with control. In the susceptible type of seedlings at 0.25% salinity the average shoot length was 1.97 at 0.50% it was reduced to 1.66 cm, at 0.75% it was 1.33 cm whereas under control condition the average shoot length of the seedlings was 5.29. In the resistant type of seedlings at 0.25% salinity the average shoot length was 3.16 cm at

0.50% it was 1.89 at 0.75% it was reduced to 0.89 cm. Thus salinity level 0.75% had an almost deleterious effect on the growth of seedlings.

Root length: The development of root was inhibited with increasing salinity level in both the susceptible and resistant type of plants. In the susceptible type of plants at 0.25% salinity the average shoot length was 0.58cm, at 0.50% it was 0.33 cm, at 0.75% it was 0.37cm. The development of root was highly inhibited and had abnormal growth with aerial, hair-like roots. In the resistant type of plants at 0.25% salinity the average shoot length was 2.05cm, at 0.50% it was reduced 1.00cm, at 0.75% it was drastically reduced to 0.25cm, whereas under control condition it was 4.86 cm. Thus salinity did inhibit the growth and development of roots but at higher salinity level i.e. at 0.75% the development of roots was almost completely inhibited.

No. of leaves: In the susceptible type of plants at 0.25% the average number of leaves was 1.40, at 0.50% it was reduced to 0.91cm and at 0.75% it was 0.74. Thus salinity had an inhibitory effect on the development of leaves. In the resistant type of plants at 0.25% the average number of leaves was 2.21, at 0.50% it was 1.04, at 0.75% it was reduced to 0.62 and under control conditions it was 2.64. Thus salinity of higher level i.e. 0.50% and 0.75% had greater inhibitory effect on the development of leaves.

Weight of plant: The average biomass production showed declining trend with the increasing salinity level in both the susceptible and resistant type of plants.

In the susceptible type of plants at 0.25% salinity the average weight of plants was 45 mg, at 0.50% it was slightly increased to 51 mg and at 0.75% it was 51 mg. In the resistant type of plants at 0.25% salinity the average weight of seedlings was 74 mg, at 0.50% it was 53 mg, at 0.75% it was reduced to 37 mg and under control conditions it was 84 mg. Thus a gradual decrease in the biomass production with increasing salinity level was observed.

ISH 5050Y

Germination: By 10th day the germination was 70%, 30%, 5% and 90% and by 20th day it was 75%, 38.3%, 8.3% and 90% at 0.25%, 0.50%, 0.75% and control conditions respectively. Thus, majority of the seeds had germinated under control and 0.25% salinity by the 10th day indicating marginal effect of 0.25% salinity.

Shoot length: In the susceptible type of plant at 0.25% salinity the average shoot length was 1.47 cm, at 0.50% it was 1.33 cm, at 0.75% it was reduced to 0.90 cm. In the resistant type of plant at 0.25% the average shoot length was 2.22 cm, at 0.50% it was 1.50 cm, at 0.75% it was reduced to 0.63 cm and under control condition it was 3.25 cm. Thus,

growth of shoot was inhibited with increasing salinity level and the inhibitory affect was more manifested at higher salinity level i.e. at 0.75%.

Root length: The growth and development of roots was highly inhibited particularly in the susceptible type of plants under varying levels of salinity as compared to the control condition. In the susceptible type of plants at 0.25% salinity the average root length was 0.18 cm, at 0.50% it was 0.32 cm, at 0.75% it was 0.23 cm. In the resistant type of plants at 0.25% the average root length was 1.65 cm, at 0.50% it was reduced to 0.93 cm, at 0.75% it was reduced to 0.18 cm and under the control condition the average root length was 2.17. Thus, the growth of root was more affected at higher salinity i.e. at 0.50% and 0.75%.

Number of leaves: In the susceptible type of plant at 0.25% salinity the average number of leaves was 1.24, at 0.50% it was again 1.24, at 0.75% it was reduced to 0.82. In the resistant type of plants at 0.25% the average number of leaves was 1.73, at 0.50% it was 1.07, at 0.75% it was reduced to 0.62 and under control condition the average number of leaves was 2.07. Thus, in both the susceptible and resistant type of plants at 0.75% salinity the development of leaves was most inhibited.

Weight of plants: In the susceptible type of plants at 0.25% the average biomass production was 31 mg, at 0.50% it was slightly increased to 48 mg, at 0.75% it was 28 mg. In the resistant type of plants at 0.25% the average biomass production was 50 mg, at 0.50% it was 47 mg, at 0.75% it was reduced to 24 mg and under control condition it was 66 mg. Thus, biomass production steadily declined with increasing salinity level except at 0.50% in the susceptible type of plants which was more as compared to 0.25% and 0.75% salinity level, though it was less as compared to the control.

ISH 34/8B

Germination: The process of germination was inhibited in the initial period of exposure to salinity as well as during the later period. By 10th day germination was 17.5%, 10%, 0% and 72.5% at 0.25%, 0.50%, 0.75% and control conditions respectively. By the 20th day 46.7%, 35%, 23.3% and 75% germination was observed at 0.25%, 0.50%, 0.75% and control conditions respectively.

Shoot length: In the susceptible type of seedlings at 0.25% salinity the average shoot length was 2.27 cm, at 0.50% it was 0.98 cm and at 0.75% it was reduced to 1.22 cm. Thus the growth of shoot was significantly inhibited under saline conditions but this inhibitory effect was more manifested at 0.50% and 0.75% salinity. In the resistant type of plants at 0.25% the average shoot length was 4.54 cm, at 0.50% it was reduced to 2.85

cm, at 0.75% it was reduced to 0.85 cm, whereas under control condition the average shoot length was 7.53 cm.

Root length: In the susceptible type of plants at 0.25% salinity the average root length was 0.40 cm, at 0.50% it was 0.22 cm, at 0.75% it was 0.51 cm. Thus, the development of roots was almost inhibited completely in the susceptible type of plants. In the tolerant type of plants the average root length was 1.85, 1.65 cm and 0.30 cm at 0.25%, 0.50%, and 0.75% salinity respectively whereas under control condition root length was 3.58 cm.

Number of leaves: In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 1.55, at 0.50% it was reduced to 1.00, at 0.75% it was 1.00. In the resistant type of seedlings at 0.25% the average number of leaves was 2.55, at 0.50% it was slightly increased to 2.99, at 0.75% it was significantly decreased to 1.00 whereas under control condition it was 3.89. Thus, at salinity level 0.75% the growth of leaves was highly inhibited.

Weight of plants: In the susceptible type of seedlings at 0.25% salinity the average biomass production was 103 mg at 0.50% it was 157 mg, at 0.75% it was 33 mg. Thus the biomass production at 0.50% was more than at 0.25% and control condition whereas at 0.75% it was significantly reduced. In the resistant type of seedlings at 0.25% the average biomass production was 83 mg, at 0.50% it was slightly increased to 103 mg, at 0.75% it was reduced to 23 mg, whereas under control condition the average weight of the seedlings was 143 mg. Thus, the biomass production at 0.75% was highly inhibited as compared to 0.25%, 0.50% and control.

ISH 34/8Y

Germination: During the initial period of exposure to saline conditions i.e. by 10th day the germination was 32.5%, 7.5%, 5% and 70% at 0.25%, 0.50%, 0.75% and control conditions respectively. By the 20th day rate of germination was 55%, 28.3%, 28.3% and 85% at 0.25%, 0.50%, 0.75% and control conditions respectively.

Shoot length: In the susceptible type of plant at 0.25% salinity the average shoot length was 3.33 cm, at 0.50% it was reduced significantly to 1.34 cm, at 0.75% it was 1.16 cm. In the resistant type of seedlings at 0.25% salinity the average shoot length was 5.20 cm, at 0.50% it was reduced significantly to 2.94 cm, at 0.75% it was 1.58 cm, whereas under control condition the average shoot length was 5.32 cm. Thus salinity did inhibit the shoot growth and this effect was more pronounced at 0.75%.

Root length: In the susceptible type of seedlings at 0.25% the average root length was 0.77 cm, at 0.50% it was 0.33 cm, at 0.75% it was 0.44 cm. The roots were aerial, hair

like and negatively geotropic. In the resistant type of seedlings at 0.25% the average root length was 3.20 cm, at 0.50% it was reduced significantly to 1.64 cm, at 0.75% it was drastically reduced to 0.99 cm and under control condition the average root length of the seedlings was 4.13. Thus, at higher salinity level 0.50% and 0.75% the growth and development of roots was significantly inhibited as compared to 0.25% and control conditions.

Number of leaves: In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 2.44, at 0.50% it was reduced to 1.00, at 0.75% it was 1.00. In the resistant type of seedlings at 0.25% the average number of leaves was 3.44, at 0.50% it was 2.22, at 0.75% it was reduced to 1.11 and under control condition the average number of leaves in the seedlings was 3.77. Thus at salinity 0.75% the growth and development of leaves was significantly reduced as compared to 0.25%, 0.50% and control conditions.

Weight of plants: The average biomass production of the seedlings increased at 0.25% salinity, as the level of salinity increased further the biomass production decreased sharply compared to control and 0.25% salinity treatment. In the susceptible type of plants at 0.25% salinity the average biomass production was 120 mg, at 0.50% it was reduced to 37 mg, at 0.75% it was 37 mg. In the resistant type of seedlings at 0.25% salinity the average biomass production was 123 mg, at 0.50% it was reduced to 70 mg, at 0.75% it was further reduced to 53 mg and under control conditions it was 110 mg. Thus, the average biomass production at 0.25% salinity was more as compared to control.

T 5-90-I

Germination: During the initial period of exposure to saline condition the germination observed was 40%, 6.6%, 0% and 93.5% at 0.25%, 0.50%, 0.75% and control conditions respectively. By the 20th day the germination was 68.3%, 36.7%, 20% and 98.3% at 0.25%, 0.50%, 0.75% and control conditions respectively.

Shoot length: The growth and development of the shoot region was more affected at salinity level 0.50% and 0.75% than at 0.25% as compared to the seedlings growing under control condition. In the susceptible type of seedlings at 0.25% salinity the average height of the seedlings was 4.03 cm, at 0.50% it was drastically reduced to 1.46 cm, at 0.75% it was further reduced to 1.02 cm. In the resistant type of seedlings at 0.25% the average shoot length was 4.55 cm, at 0.50% it was significantly reduced to 2.51 cm, at 0.75% it was further reduced to 1.04 cm and under control condition it was 6.70 cm. Thus, the growth of shoot was significantly inhibited at salinity above 0.25%.

Root length: The growth and development of roots were significantly inhibited at salinity level above 0.25% when compared to control. In the susceptible type of seedlings at 0.25% the average root length was 3.07 cm, at 0.50% it was highly inhibited to 0.55 cm, at 0.75% it was only 0.43 cm. In the resistant type of seedlings at 0.25% the average root length was 3.75 cm, at 0.50% it was significantly inhibited to 1.63 cm, at 0.75% the growth of root was almost completely inhibited and only 0.57 cm long roots could develop, whereas under control condition the average root length of the seedlings was 4.58 cm. Thus the growth and development of roots was highly inhibited under higher salinity treatments.

Number of leaves: In the susceptible type of seedlings at 0.25% salinity the average number of leaves was 2.99, at 0.50% it was reduced to 1.17, at 0.75% it was further reduced to 1.0. In the resistant type of plants at 0.25% salinity the average number of leaves was 3.11, at 0.50% it was significantly reduced to 1.55, at 0.75% it was 1.22 and under control condition the average number of leaves in the seedlings was 3.66. Thus salinity above 0.25% had a more inhibitory effect on the growth and development of leaves.

Weight of plants: In the susceptible type of seedlings at 0.25% the average biomass production was 123 mg, 0.50% it was reduced to 80 mg, at 0.75% it was substantially reduced to 27 mg. In the resistant type of seedlings at 0.25% salinity the average biomass production was 147 mg, at 0.50% it was reduced to 107 mg, at 0.75% it was substantially reduced to 40 mg and under control conditions it was 140 mg.

T 9-90-FM

Germination: During the initial period of exposure to salinity the germination was 43.3%, 17.5%, 0% and 91.6% at 0.25%, 0.50%, 0.75% and control conditions respectively. By 20th day the rate of germination was 85%, 61.7%, 16.7% and 95% at 0.25%, 0.50%, 0.75% and control conditions respectively. Thus higher levels of salinity reduced the rate of germination significantly.

Shoot length: The average shoot length in the susceptible type of plants at 0.25% salinity was 2.20 cm which was reduced to 1.34 and 1.01 cm at 0.50% and 0.75% salinity respectively. In the resistant type of plants at 0.25% salinity the average shoot length was 5.62 cm which was significantly reduced to 1.92 cm at 0.50% salinity and at 0.75% salinity it was further reduced to 0.90 cm whereas under control condition the average shoot length of the plants was 7.17 cm. Thus higher salinity levels had high inhibitory effect on the growth of shoot portion.

Root length: The average root length of the susceptible type of plants growing at 0.25%, 0.50% and 0.75% salinity was 0.56 cm, 0.33 cm and 0.30 cm. In the resistant type of plants it was 3.54cm, 1.93 cm and 0.33 cm at 0.25%, 0.50% and 0.75% salinity respectively. The average root length of the plants growing under control condition was 3.90 cm. Thus the growth of roots was almost completely inhibited in the susceptible type of plants at all salinity levels whereas in the resistant plants higher salinity levels inhibited the growth of roots to greater extent.

Number of leaves: In the susceptible type of seedlings the average number of leaves was 1.33, 1.33 and 1.0 at 0.25%, 0.50% and 0.75% salinity respectively. In the tolerant seedlings it was 3.78, 1.11 and 1.0cm at 0.25%, 0.50% and 0.75% salinity respectively whereas under control condition the average number of leaves was 3.77. Thus the average number of leaves was reduced with increasing levels of salinity.

Weight of plants: The average biomass production in the susceptible type of plants at 0.25% salinity was 87mg which was further reduced to 80mg and 40mg at 0.50% and 0.75% salinity. In the tolerant seedlings at 0.25% salinity the average biomass production was 150 mg which was reduced to 87 mg at 0.50% salinity but at 0.75% salinity the biomass production was substantially increased to 367 mg whereas under control condition the average biomass production of the seedlings was 153 mg.

A.2. Analysis of variance

A.2.1. Single factor analysis

Shoot length: ANOVA analysis revealed significant differences for shoot length under four salinity treatments among genotypes in susceptible group of plants. Genotype Penta 99-1 showed non-significant variation for shoot length (table). However, among resistant type of plants all the genotypes showed highly significant ($p \leq 0.01$) differences for shoot length.

Root length: The root lengths of the susceptible as well as resistant type of plants among genotypes under different salinity treatments were found to be significant ($p \leq 0.05$) except in the genotype EC 407709 where it was non significant ($p \geq 0.05$) in susceptible group and significant in resistant group.

Number of leaves: The number of leaves in the susceptible type of plants among genotypes was found to be significant ($p \leq 0.05$) except in the genotypes Raj Bundi, Penta 99-1, ISH 32/8/1, and T 45-1 wherein it was found to be non –significant ($p \geq 0.05$). Among resistant type of plants, the number of leaves among genotypes was found to be

significant ($p \leq 0.05$) except in the genotypes EC 407709, ISH 34/49, ISH 32/8/1 and ISH 8020B where it was found to be non- significant.

Plant weight: The weight of the susceptible type of plants was significant ($p \leq 0.05$) under the four salinity treatments except the genotypes EC 407709, EC 4017103, ISH 34/49, ISH 34/41, ISH 34/11, Penta 99, Raj Bundi, T 45-1, ISH 5050B, and T-9-90FM wherein it was found to be non-significant ($p \geq 0.05$). The weight of the resistant type of plants in the genotypes was significant under the 4 salinity treatments except in EC 318954, EC 4017103, ISH 34/49, ISH 34/11, Penta 99, Raj Bundi and Penta 99-1 where it was found to be non- significant.

Germination (percent): The percent germination among genotypes evaluated was significant under the 4 salinity treatments.

A.2.2. Two factor analysis

Susceptible shoot length: The effect of genotypes, salinity level treatments and their interaction for shoot length in the susceptible plants was found to be highly significant ($p \leq 0.01$).

Resistant shoot length: The effect of genotypes, salinity level treatments and their interaction for shoot length in the resistant plants was found to be highly significant.

Susceptible root length: The effect of genotypes, salinity level treatments and their interaction for root length in the susceptible group of plants was found to be highly significant.

Resistant root length: The effect of genotypes, salinity level treatments and their interaction for root length in the resistant plants was found to be highly significant.

Number of leaves susceptible: The effect of genotypes, salinity level treatments and their interaction for number of leaves in the susceptible type of plants was found to be highly significant.

Number of leaves Resistant: The effect of genotypes, salinity level treatments and their interaction for number of leaves in the resistant plant types was found to be highly significant.

Plant weight, susceptible: The effect of genotypes, different levels of salinity treatments and their interaction for weight of plants in the susceptible type was found to be highly significant.

Table 7. Germination, shoot length and root length (20 days old seelding) of Egyptian clover genotypes growing *in vitro*

Genotype	% germination				Shoot length (cm)								Root length (cm)							
					Sus				Resis				Sus				Resis			
	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control
EC 329299	90.0	68.3	26.7	94.3	5.55	0.80	0.96	6.70	5.94	3.25	1.34	7.01	5.14	1.47	0.27	5.86	5.76	2.45	0.98	6.41
EC 318954	90.0	86.7	38.3	96.0	5.58	1.59	1.06	7.03	5.95	3.04	1.66	7.50	5.36	0.34	0.11	6.79	6.13	2.98	0.84	7.49
Wardan	68.7	36.7	18.3	75.0	0.83	0.83	0.85	3.52	2.55	1.45	0.90	3.76	0.91	0.34	0.28	2.74	2.21	0.72	0.27	2.97
EC 407709	83.3	81.7	75.0	95.0	3.45	4.17	2.58	10.61	8.84	11.36	10.72	12.50	1.89	2.88	2.81	4.73	4.08	4.69	3.95	5.47
EC400976	76.7	50.0	13.3	90.0	2.28	1.64	1.39	6.71	6.25	2.51	1.51	8.21	2.68	2.29	1.63	5.91	4.45	2.76	1.56	7.11
EC 508311	16.7	11.7	0.0	70.0	2.21	1.78	0.00	4.88	2.20	1.92	0.00	5.20	1.28	2.11	0.00	2.66	1.43	2.08	0.00	2.94
EC4017103	28.3	13.3	8.3	57.3	2.16	1.14	1.09	4.50	2.28	1.14	1.26	4.83	1.09	0.44	0.48	1.97	1.08	0.48	0.52	2.20
EC400977	51.7	8.3	10.0	55.7	1.62	1.03	1.43	4.13	1.80	0.95	1.54	4.46	1.18	0.64	0.95	2.22	1.22	0.63	0.90	2.40
EC401711	36.7	16.7	6.7	56.3	1.73	1.87	0.67	5.50	4.19	3.92	0.80	6.29	1.17	1.03	0.68	3.94	2.44	2.18	0.69	4.23
ISH 34/49	90.0	70.0	33.3	95.0	1.76	2.03	1.49	4.06	3.60	2.43	2.72	3.66	0.72	0.90	0.72	2.24	2.02	1.75	1.15	2.54
ISH 34/41	56.7	46.7	43.3	90.0	3.19	1.99	1.10	4.57	2.72	1.52	0.90	4.05	0.54	0.36	0.31	2.29	0.60	0.38	0.43	2.35
ISH 34/11	95.0	75.0	71.7	93.3	2.25	1.74	1.52	3.34	2.40	2.11	1.36	3.08	1.52	0.91	0.68	2.65	1.63	1.56	0.72	2.97
Penta 99	63.3	65.0	31.7	70.0	1.67	1.71	1.25	3.03	4.10	2.40	1.29	3.21	0.60	0.72	0.79	2.01	1.74	1.29	0.74	2.31
Raj Bundi	58.3	45.0	35.0	71.7	2.47	1.98	1.71	3.79	3.54	1.77	1.73	4.17	0.89	0.79	0.84	2.50	2.33	1.83	0.84	2.69
Penta 99-1	61.7	46.7	45.0	72.0	2.33	2.43	1.57	2.82	2.71	1.67	1.62	3.25	1.94	1.02	0.78	2.66	2.10	2.57	0.71	2.73
ES 99	66.7	43.3	21.7	83.3	2.87	2.58	1.64	4.87	4.02	3.08	1.77	5.50	1.20	1.68	0.82	4.24	3.82	3.02	0.82	4.26
ISH 32/8/1	48.3	31.7	20.0	85.0	2.40	1.93	1.43	3.15	2.51	1.99	1.49	3.34	1.38	1.18	0.60	3.18	1.39	1.17	0.52	3.46
Wardan S2	61.7	38.3	28.3	86.7	1.71	1.72	0.71	4.13	1.89	2.27	0.73	4.47	0.60	0.40	0.37	2.26	0.65	1.52	0.39	2.59
ISH 26/50/7	41.7	20.0	13.3	91.7	1.04	1.07	0.45	3.73	3.08	1.11	0.45	4.21	0.46	0.58	0.27	3.14	1.81	0.54	0.33	3.58
ISH 32/34/1	63.3	18.3	6.7	76.7	1.05	1.01	0.37	3.28	3.05	2.80	0.38	3.71	0.64	0.39	0.39	2.23	1.52	2.09	0.33	2.51
Multi-98-45	48.3	16.7	11.7	80.0	1.62	0.72	0.64	3.68	1.70	0.74	0.55	3.95	0.85	0.56	0.30	2.92	1.45	0.57	0.26	3.00
ISH 34/5/1	65.0	33.3	11.7	76.7	1.14	0.93	0.49	2.88	1.96	1.24	0.41	3.29	0.69	0.43	0.21	2.26	1.48	1.40	0.31	2.37
Raj 49/50	8.3	0.0	0.0	45.0	0.75	0.00	0.00	2.45	0.62	0.00	0.00	2.71	0.66	0.00	0.00	1.98	0.59	0.00	0.00	2.35
T 5-90I-1	46.7	10.0	10.0	88.3	1.41	0.29	1.39	2.84	1.38	0.27	1.25	3.23	0.36	0.19	0.36	1.93	2.30	0.13	0.26	3.13
T 45-1	53.3	16.7	15.0	100.0	1.72	1.14	1.16	4.14	2.57	2.31	0.97	5.10	1.01	0.19	0.73	3.79	2.30	0.94	0.60	4.52
T 44-4	51.7	16.7	16.7	85.0	1.28	1.00	1.08	4.13	2.73	0.82	0.97	4.76	1.80	0.50	0.74	6.18	1.68	0.39	0.54	8.03
ISH 8020B	68.3	46.7	25.0	98.3	0.81	1.59	0.98	6.52	3.67	2.31	1.71	5.98	1.23	0.53	0.38	5.07	4.40	1.67	0.55	5.51
ISH 8020Y	56.7	30.0	18.3	91.7	2.20	1.11	1.02	5.50	2.79	1.98	0.83	6.25	0.35	0.23	0.35	4.21	1.98	0.87	0.26	4.95
ISH 5050B	80.0	33.3	38.3	93.3	1.97	1.66	1.33	5.29	3.16	1.89	0.89	4.66	0.58	0.33	0.37	4.54	2.05	1.00	0.25	4.86
ISH 5050Y	75.0	38.3	8.3	90.0	1.47	1.33	0.90	3.46	2.22	1.50	0.63	3.25	0.18	0.32	0.23	2.31	1.65	0.93	0.18	2.17
ISH 34/8B	46.7	35.0	23.3	75.0	2.27	0.98	1.22	6.43	4.54	2.85	0.85	7.53	0.40	0.22	0.51	2.84	1.85	1.65	0.30	3.58
ISH 34/8Y	55.0	28.3	28.3	85.0	3.33	1.34	1.16	4.03	5.20	2.94	1.58	5.32	0.77	0.33	0.44	3.77	3.20	1.64	0.99	4.13
T 5-90-I	68.3	36.7	20.0	98.3	4.03	1.46	1.02	5.63	4.55	2.51	1.04	6.70	3.07	0.55	0.43	4.19	3.75	1.63	0.57	4.58
T-9-90FM	85.0	61.7	16.7	95.0	2.20	1.34	1.01	6.13	5.62	1.92	0.90	7.17	0.56	0.33	0.30	3.32	3.54	1.93	0.33	3.90
	Sus=Susceptible			Resis=resistant																

Table 8. Number of leaves and biomass of Egyptian clover genotype 20 days old seedlings growing *in vitro*

Genotype	No of leaves								Weight of plant (g)							
	Sus				Resis				Sus				Resis			
	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control
EC 329299	3.50	2.00	1.18	3.93	3.74	2.60	1.39	4.13	0.144	0.044	0.029	0.132	0.152	0.090	0.040	0.149
EC 318954	3.21	1.07	1.07	4.14	3.20	1.85	1.39	4.13	0.109	0.047	0.044	0.200	0.118	0.068	0.186	0.217
Wardan	1.29	1.18	1.07	2.14	1.71	1.25	1.07	2.35	0.038	0.032	0.026	0.065	0.062	0.037	0.025	0.068
EC 407709	2.86	2.14	2.14	6.14	5.42	4.96	5.81	6.00	0.116	0.069	0.055	0.148	0.107	0.161	0.142	0.161
EC400976	2.36	2.14	2.14	6.14	5.35	3.92	1.96	5.61	0.060	0.037	0.042	0.152	0.151	0.112	0.049	0.166
EC 508311	2.14	2.14	0.00	3.71	1.96	1.96	0.00	3.24	0.037	0.042	0.000	0.060	0.039	0.039	0.000	0.073
EC4017103	1.65	1.65	1.65	2.64	1.72	1.72	1.72	2.78	0.045	0.039	0.026	0.336	0.061	0.053	0.030	0.121
EC400977	1.65	1.65	1.65	2.00	1.72	1.72	1.72	2.23	0.032	0.032	0.019	0.052	0.038	0.038	0.030	0.068
EC401711	1.65	1.65	1.65	2.14	2.49	1.72	1.72	2.26	0.058	0.058	0.026	0.110	0.129	0.114	0.030	0.129
ISH 34/49	0.86	1.31	0.94	1.91	1.64	1.58	2.02	2.14	0.038	0.038	0.035	0.044	0.047	0.047	0.040	0.043
ISH 34/41	2.80	1.20	0.86	2.14	1.99	1.25	0.98	2.62	0.071	0.056	0.035	0.065	0.043	0.053	0.037	0.084
ISH 34/11	1.11	0.86	0.86	1.89	1.07	0.98	0.98	2.35	0.032	0.032	0.029	0.035	0.031	0.031	0.028	0.034
Penta 99	1.40	0.84	1.20	1.85	1.75	1.01	1.38	2.22	0.044	0.044	0.050	0.053	0.050	0.053	0.050	0.056
Raj Bundi	1.54	1.57	1.09	1.85	2.02	1.01	1.28	2.32	0.074	0.106	0.068	0.059	0.050	0.050	0.056	0.059
Penta 99-1	1.58	1.68	1.12	1.93	2.02	1.11	1.52	2.47	0.121	0.121	0.129	0.062	0.062	0.050	0.437	0.062
ES 99	1.79	1.61	1.61	3.38	2.35	1.77	1.77	3.53	0.080	0.094	0.040	0.104	0.068	0.063	0.044	0.107
ISH 32/8/1	1.61	1.61	1.61	1.79	1.94	1.77	1.77	1.96	0.070	0.055	0.035	0.070	0.068	0.054	0.034	0.073
Wardan S2	1.30	1.12	0.84	2.24	1.36	1.76	0.97	2.65	0.035	0.042	0.032	0.067	0.041	0.059	0.032	0.066
ISH 26/50/7	1.26	1.21	0.92	2.41	1.87	0.97	0.88	2.95	0.032	0.042	0.017	0.062	0.041	0.041	0.017	0.068
ISH 32/34/1	1.26	0.93	0.93	2.23	1.57	1.95	0.88	2.36	0.037	0.027	0.017	0.057	0.059	0.073	0.017	0.061
Multi-98-45	1.12	0.97	0.88	2.34	0.81	0.81	0.81	2.26	0.048	0.022	0.020	0.065	0.049	0.019	0.014	0.071
ISH 34/5/1	1.26	1.20	0.97	2.33	1.17	0.90	0.81	2.26	0.059	0.042	0.022	0.051	0.044	0.046	0.019	0.055
Raj 49/50	0.88	0.00	0.00	1.56	0.81	0.00	0.00	1.63	0.022	0.000	0.000	0.045	0.022	0.000	0.000	0.046
T 5-90I-1	1.08	0.75	0.75	1.74	0.92	0.76	0.76	2.06	0.058	0.017	0.034	0.071	0.090	0.012	0.022	0.068
T 45-1	1.49	2.15	0.83	1.98	1.51	1.34	0.76	2.35	0.095	0.058	0.041	0.068	0.062	0.053	0.031	0.078
T 44-4	0.75	0.75	0.75	2.32	1.51	0.76	0.84	2.43	0.058	0.048	0.027	0.129	0.087	0.037	0.028	0.152
ISH 8020B	0.91	0.74	0.94	3.05	1.86	1.52	1.35	2.76	0.042	0.056	0.039	0.118	0.087	0.060	0.042	0.144
ISH 8020Y	2.47	0.82	0.82	1.98	1.73	1.11	0.62	2.07	0.070	0.037	0.037	0.081	0.058	0.042	0.032	0.100
ISH 5050B	1.40	0.91	0.74	2.64	2.21	1.04	0.62	2.62	0.045	0.051	0.051	0.073	0.074	0.053	0.037	0.084
ISH 5050Y	1.24	1.24	0.82	2.06	1.73	1.07	0.62	2.07	0.031	0.048	0.028	0.056	0.050	0.047	0.024	0.066
ISH 34/8B	1.55	1.00	1.00	2.89	2.55	2.99	1.00	3.89	0.103	0.157	0.033	0.097	0.083	0.103	0.023	0.143
ISH 34/8Y	2.44	1.00	1.00	3.66	3.44	2.22	1.11	3.77	0.120	0.037	0.037	0.097	0.123	0.070	0.053	0.110
T 5-90-I	2.99	1.17	1.00	3.44	3.11	1.55	1.22	3.66	0.123	0.080	0.027	0.130	0.147	0.107	0.040	0.140
T-9-90FM	1.33	1.33	1.00	3.33	3.78	1.11	1.00	3.77	0.087	0.080	0.040	0.133	0.150	0.087	0.367	0.153
	Sus=Susceptible			Resis=resistant												

Table 9. Single factor analysis of variance for various traits in 20 days old seedling of Egyptian clover genotypes.									
	Shoot length		Root length		No. of leaves		Weight of plant		% Germination
	S	R	S	R	S	R	S	R	
Genotypes	*MS*	*MS*	*MS*	*MS*	*MS*	*MS*	*MS*	*MS*	*MS*
EC 329299	28.189**	19.847**	22.426**	20.392**	4.944**	4.571**	0.01**	0.009**	2872.556**
EC 318954	26.011**	21.309**	35.281**	27.237**	7.227**	4.723**	0.016**	0.014*	2116.306**
Wardan	5.381**	4.804**	3.954**	4.777**	0.72**	0.981**	0.001**	0.001**	2080.556**
EC 407709	40.308**	7.028**	4.25**	1.441*	10.956**	0.639*	0.005*	0.002**	207.639**
EC400976	18.689**	29.691**	10.916**	17.325**	11.607**	8.43**	0.009**	0.008**	3430.556**
EC 508311	12.22**	13.837**	4.016**	4.603**	6.981**	5.355**	0.002**	0.003**	2896.528**
EC4017103	7.652**	8.804**	1.527**	1.933**	0.733**	0.843**	0.068*	0.005*	1457**
EC400977	5.904**	7.296**	1.413**	1.832**	0.093**	0.199**	0.001**	0.001**	1989.639**
EC401711	13.287**	15.357**	6.799**	6.316**	0.183	0.458**	0.004**	0.007**	1456.75**
ISH 34/49	4.121**	1.156**	1.63**	1.003**	0.694**	0.235*	0*	0*	2352.083**
ISH 34/41	6.809**	5.805**	2.687**	2.662**	2.356**	1.645**	0.001**	0.001**	1363.889**
ISH 34/11	1.962**	1.521**	2.32**	2.596**	0.711**	1.348**	0*	0*	440.972**
Penta 99	1.78**	4.274**	1.293**	1.336**	0.529**	0.806**	0*	0*	913.889**
Raj Bundi	2.571**	4.621**	2.065**	1.939**	0.293*	1.137**	0.001*	0*	763.889**
Penta 99-1	0.826**	1.919**	2.238**	2.516**	0.348*	1.047**	0.003**	0.107*	495.778**
ES 99	5.533**	7.405**	7.141**	7.005**	2.222**	2.079**	0.002**	0.002**	2179.861**
ISH 32/8/1	1.611**	1.878**	3.734**	4.846**	0.024*	0.035*	0.001**	0.001**	2407.639**
Wardan S2	6.341**	7.309**	2.466**	2.964**	1.101**	1.559**	0.001**	0.001**	2029.861**
ISH 26/50/7	6.463**	9.057**	5.548**	6.692**	1.287**	2.77**	0.001**	0.001**	3772.222**
ISH 32/34/1	4.864**	6.332**	2.369**	2.674**	1.139**	1.172**	0.001**	0.002**	3463.194**
Multi-98-45	5.991**	7.3**	4.293**	4.511**	1.392**	1.563**	0.001**	0.002**	3013.889**
ISH 34/5/1	3.318**	4.483**	2.602**	2.133**	1.107**	1.331**	0.001**	0.001**	2638.889**
Raj 49/50	4.006**	4.955**	2.61**	3.719**	1.713**	1.817**	0.001**	0.001**	1383.333**
T 5-90I-1	3.284**	4.582**	1.993**	6.7**	0.656**	1.195**	0.002**	0.004**	4174.306**
T 45-1	6.116**	8.902**	7.734**	9.456**	1.061*	1.298**	0.002*	0.001**	4790.972**
T 44-4	6.836**	10.16**	20.97**	39.455**	1.852**	1.805**	0.006**	0.01**	3225**
ISH 8020B	22.195**	10.742**	14.654**	15.999**	3.612**	1.191**	0.004**	0.006**	2940.972**
ISH 8020Y	13.222**	16.344**	11.427**	13.003**	2.098**	1.247**	0.002**	0.003**	3180.556**
ISH 5050B	10.137**	7.993**	12.743**	12.251**	2.205**	2.69**	0**	0.001**	2685.417**
ISH 5050Y	3.895**	3.704**	3.229**	2.266**	0.806**	1.264**	0.001**	0.001**	4063.194**
ISH 34/8B	19.283**	23.984**	4.602**	5.424**	2.377**	4.367**	0.008*	0.008**	1472.222**
ISH 34/8Y	6.168**	9.921**	8.039**	6.166**	4.96**	4.442**	0.005*	0.003**	2186.111**
T 5-90-I	14.292**	18.176**	10.452**	10.302**	4.654**	4.209**	0.007**	0.007**	3613.889**
T-9-90 FM	16.731**	26.593**	6.445**	8.029**	3.406**	7.404**	0.004*	0.045**	3646.528**
	*= p<0.05, **=p<0.01								
	Degree of freedom=3		S= Susceptible		R= resistant				

Table 10. Two factor analysis of variance for various traits in 20 days old seedling of Egyptian clover genotypes.

	SS	df	MS
Shoot length (S).			
Genotype	255.037	33	7.728**
Treatment	777.885	3	259.295**
Interaction	230.103	99	2.324**
Shoot length (R)			
Genotype	1080.612	33	32.746**
Treatment	782.831	3	260.944**
Interaction	228.439	99	2.307**
Root length (S)			
Genotype	204.706	33	6.203**
Treatment	520.442	3	173.481**
Interaction	187.158	99	1.89**
Root length (R)			
Genotype	362.699	33	10.991**
Treatment	562.654	3	187.551**
Interaction	221.843	99	2.241**
No. of leaves (S)			
Genotype	123.707	33	3.749**
Treatment	162.317	3	54.106**
Interaction	95.816	99	0.968**
No.of leaves (R)			
Genotype	362.699	33	10.991**
Treatment	562.654	3	187.551**
Interaction	221.843	99	2.241**
Plant weight (S)			
Genotype	0.243	33	0.007**
Treatment	0.180	3	0.06**
Interaction	0.330	99	0.003**
Plant weight (R)			
Genotype	0.627	33	0.019**
Treatment	0.091	3	0.03**
Interaction	0.680	99	0.007**
Germination %			
Genotype	101592.787	33	3078.569**
Treatment	207699.694	3	69233.231**
Interaction	37418.556	99	377.965**
R= Resistant, S=Susceptible		*=p<0.05, **=p<0.01	

Fig. 2.1

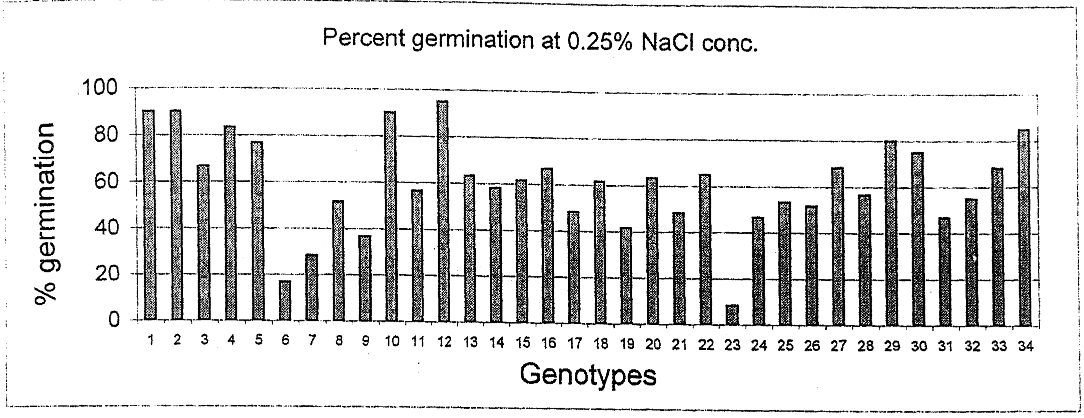


Fig. 2.2

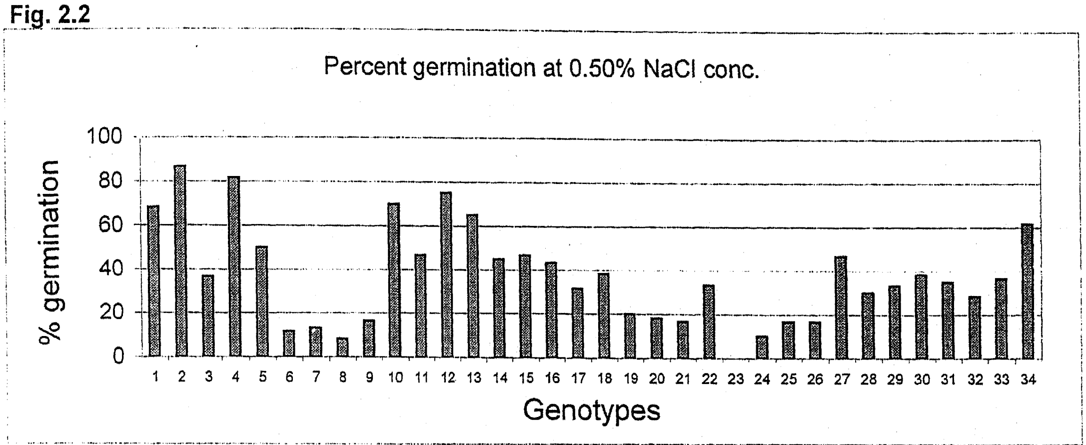


Fig. 2.3

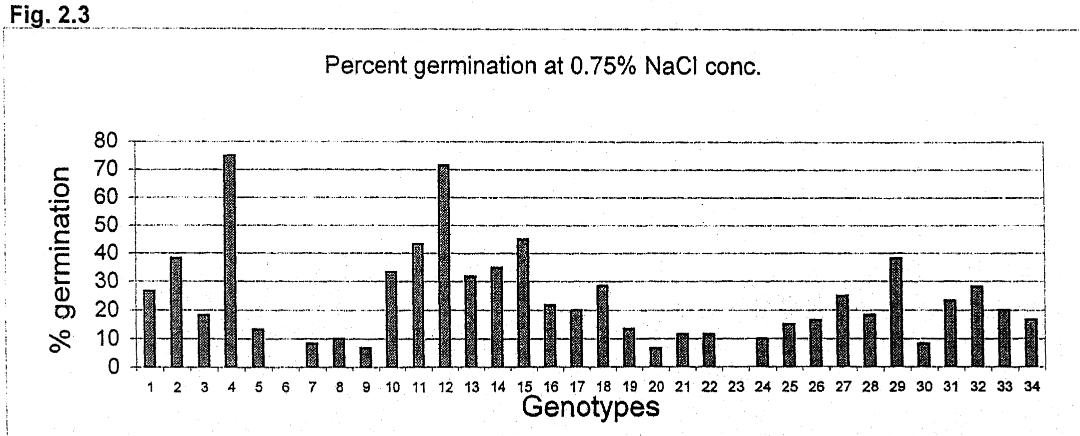
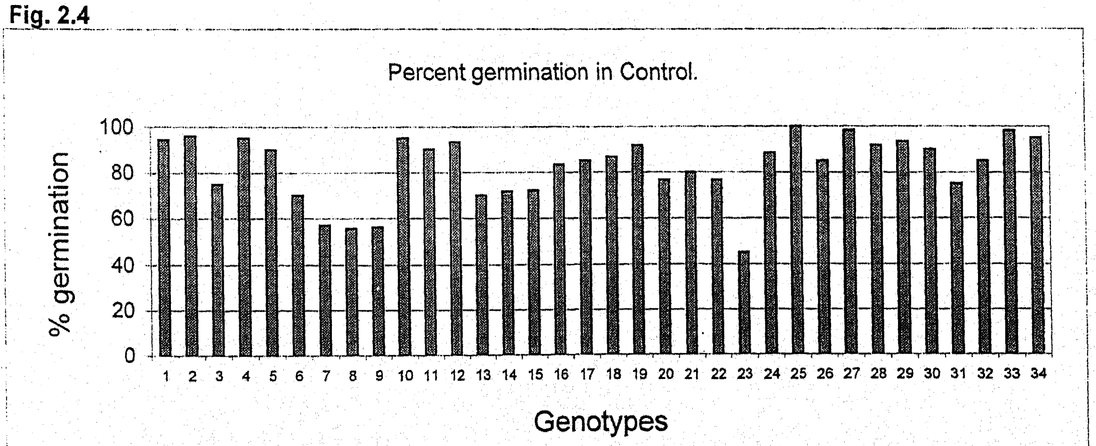


Fig. 2.4



1. EC 329299, **2.** EC 318954, **3.** Wardan, **4.** EC 407709, **5.** EC 400976, **6.** EC 508311, **7.** EC 4017103, **8.** EC 400977, **9.** EC 401711, **10.** ISH 34/49, **11.** ISH 34/41, **12.** ISH 34/11, **13.** Penta 99, **14.** Raj Bundi, **15.** Penta 99-1, **16.** ES 99, **17.** ISH 32/8/1, **18.** Wardan S2, **19.** ISH 26/50/7, 20. ISH 32/34/1, **21.** Multi-98-45, **22.** ISH 34/5/1, **23.** Raj 49/50, **24.** T 5-90I-1, **25.** T 45-1, **26.** T 44-4, **27.** ISH 8020B, **28.** ISH 8020Y, **29.** ISH 5050B, **30.** ISH 5050Y, **31.** ISH 34/8B, **32.** ISH 34/8Y, **33.** T 5-90-I, **34.** T-9-90FM.

Fig. 3.1

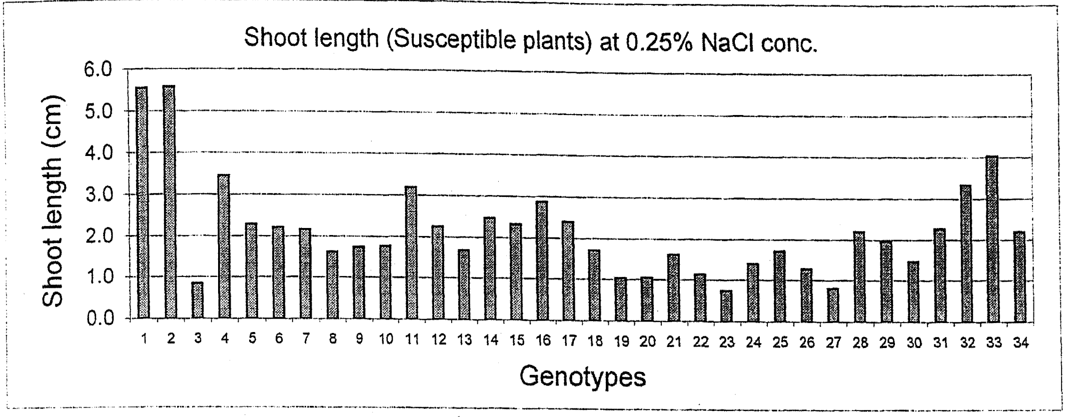


Fig. 3.2

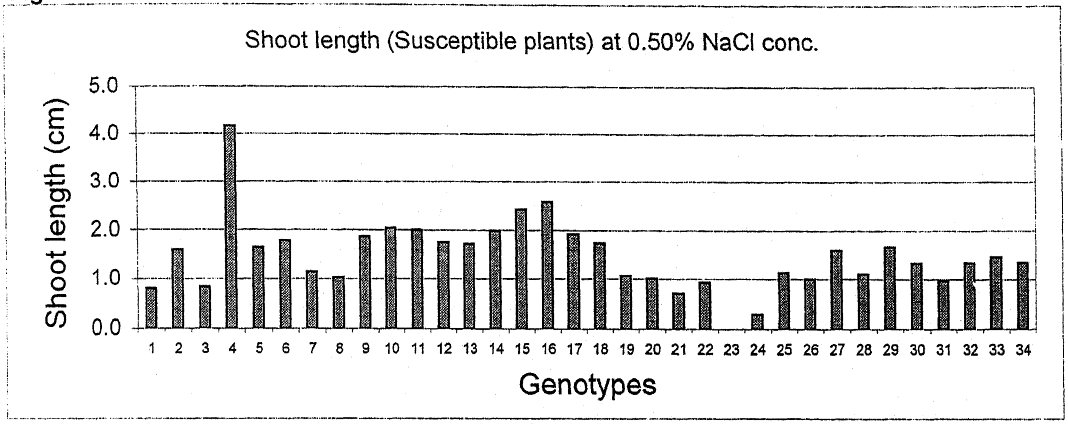


Fig. 3.3

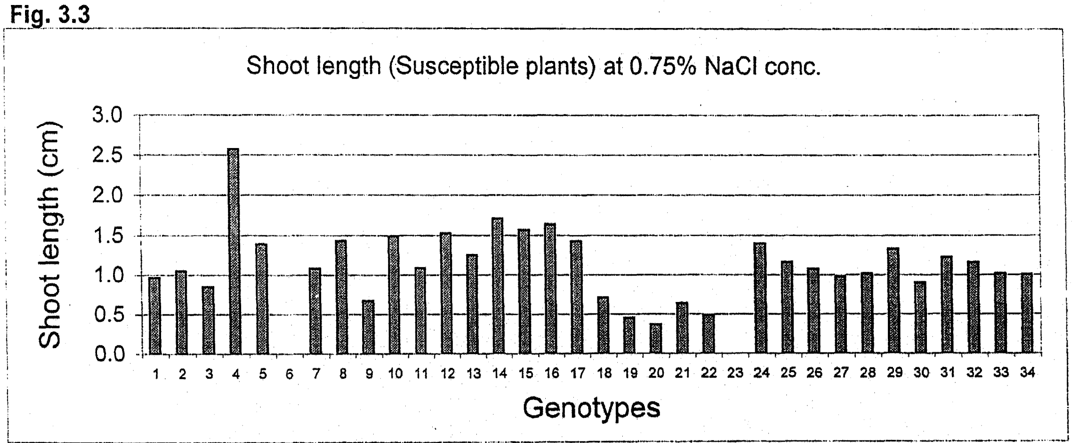
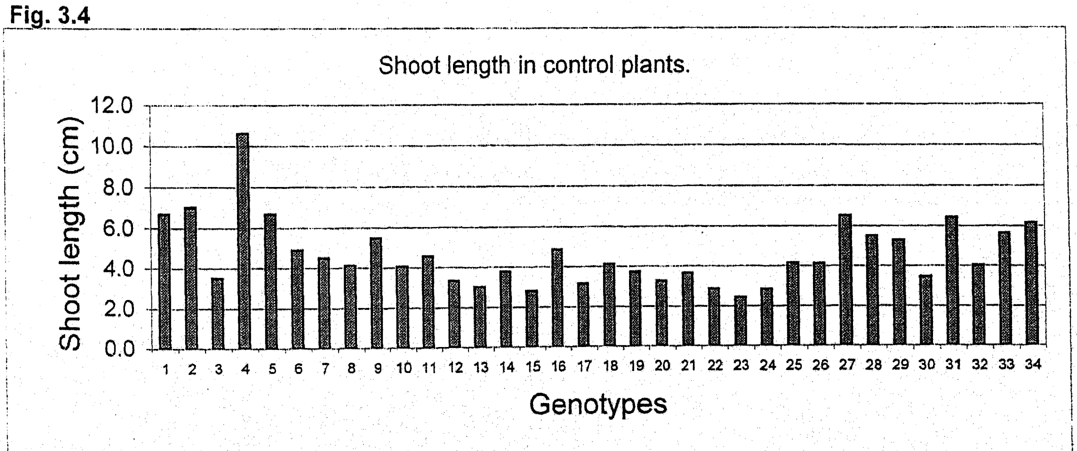


Fig. 3.4



1. EC 329299, 2. EC 318954, 3. Wardan, 4. EC 407709, 5. EC 400976, 6. EC 508311, 7. EC 4017103, 8. EC 400977, 9. EC 401711, 10. ISH 34/49, 11. ISH 34/41, 12. ISH 34/11, 13. Penta 99, 14. Raj Bundi, 15. Penta 99-1, 16. ES 99, 17. ISH 32/8/1, 18. Wardan S2, 19. ISH 26/50/7, 20. ISH 32/34/1, 21. Multi-98-45, 22. ISH 34/5/1, 23. Raj 49/50, 24. T 5-90!-1, 25. T 45-1, 26. T 44-4, 27. ISH 8020B, 28. ISH 8020Y, 29. ISH 5050B, 30. ISH 5050Y, 31. ISH 34/8B, 32. ISH 34/8Y, 33. T 5-90-I, 34. T-9-90FM.

Fig. 3.5

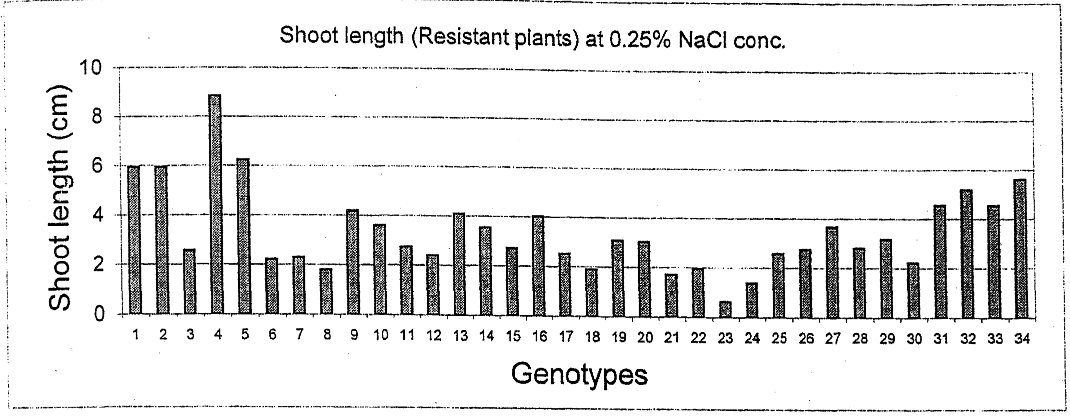


Fig. 3.6

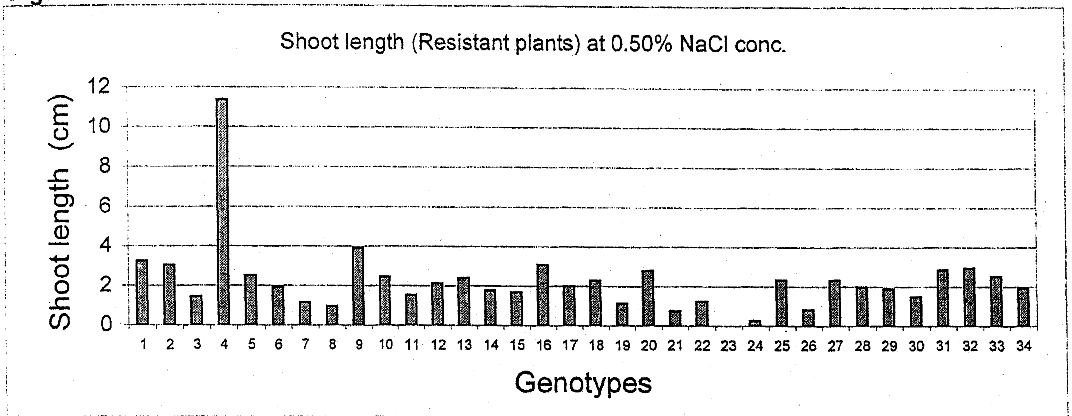


Fig. 3.7

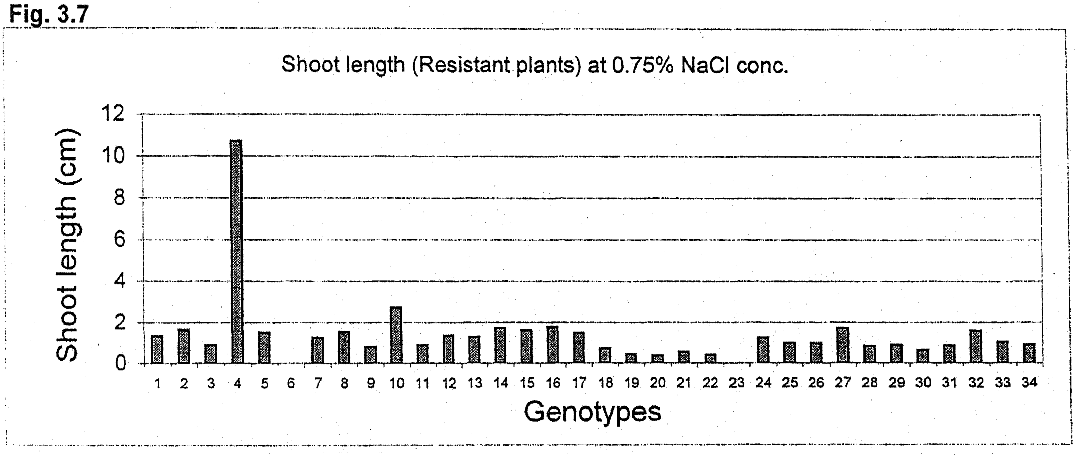
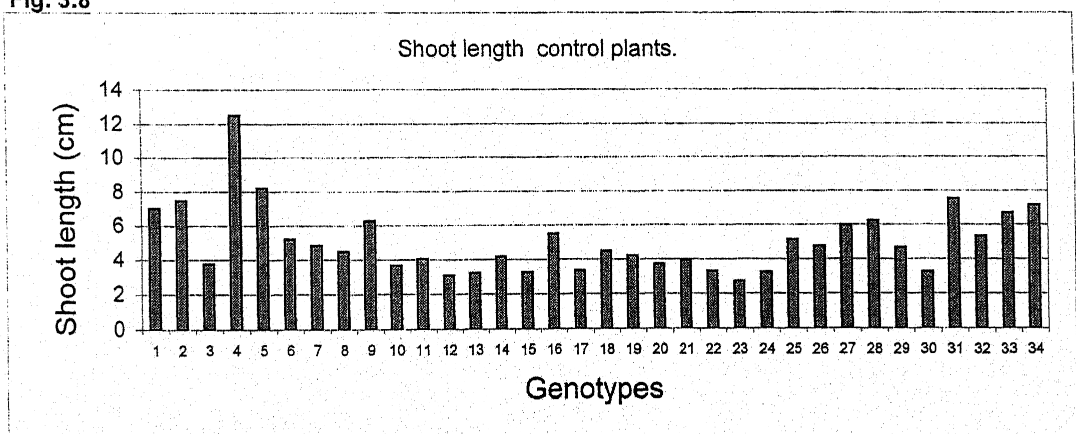


Fig. 3.8



1. EC 329299, 2. EC 318954, 3. Wardan, 4. EC 407709, 5. EC 400976, 6. EC 508311, 7. EC 4017103, 8. EC 400977, 9. EC 401711, 10. ISH 34/49, 11. ISH 34/41, 12. ISH 34/11, 13. Penta 99, 14. Raj Bundi, 15. Penta 99-1, 16. ES 99, 17. ISH 32/8/1, 18. Wardan S2, 19. ISH 26/50/7, 20. ISH 32/34/1, 21. Multi-98-45, 22. ISH 34/5/1, 23. Raj 49/50, 24. T 5-90I-1, 25. T 45-1, 26. T 44-4, 27. ISH 8020B, 28. ISH 8020Y, 29. ISH 5050B, 30. ISH 5050Y, 31. ISH 34/8B, 32. ISH 34/8Y, 33. T 5-90-I, 34. T-9-90FM.

Fig. 4.1

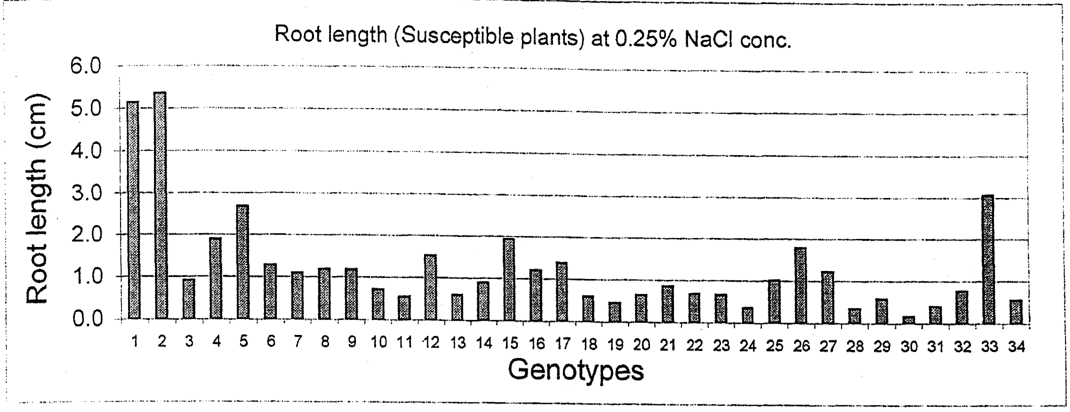


Fig. 4.2

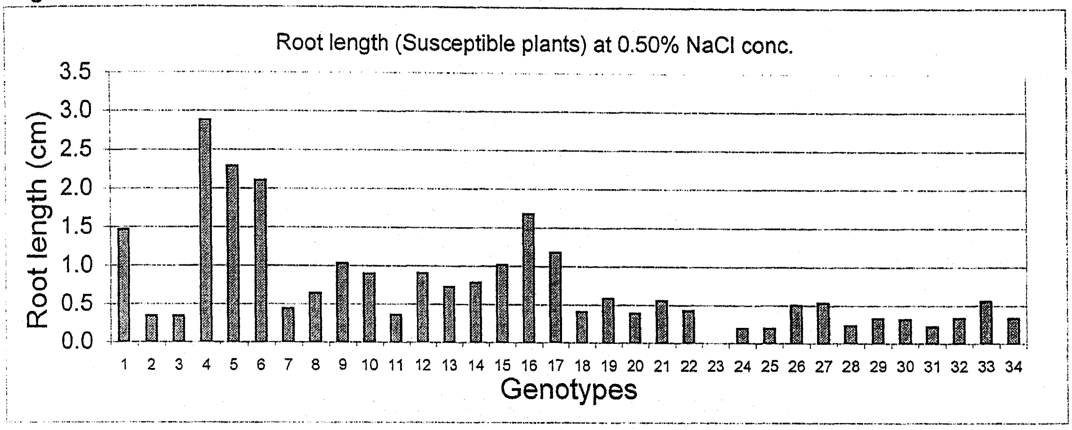


Fig. 4.3

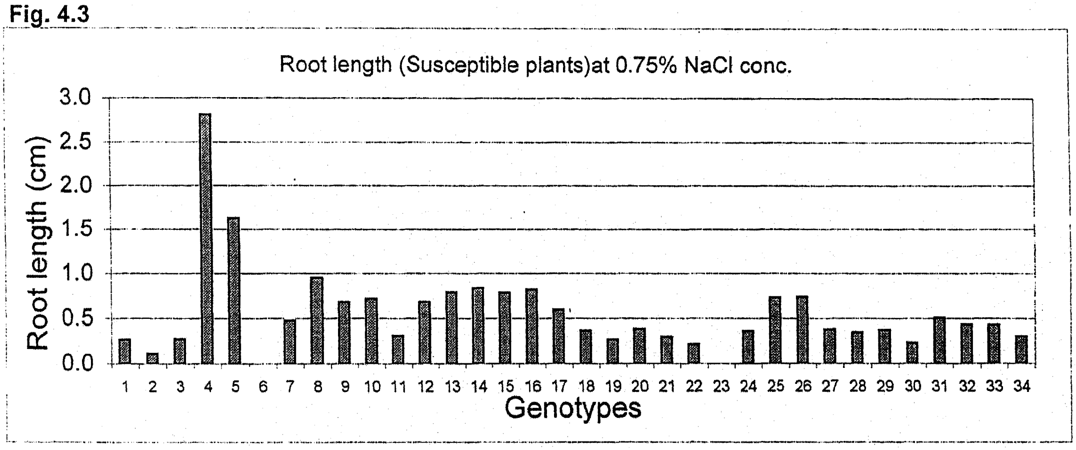
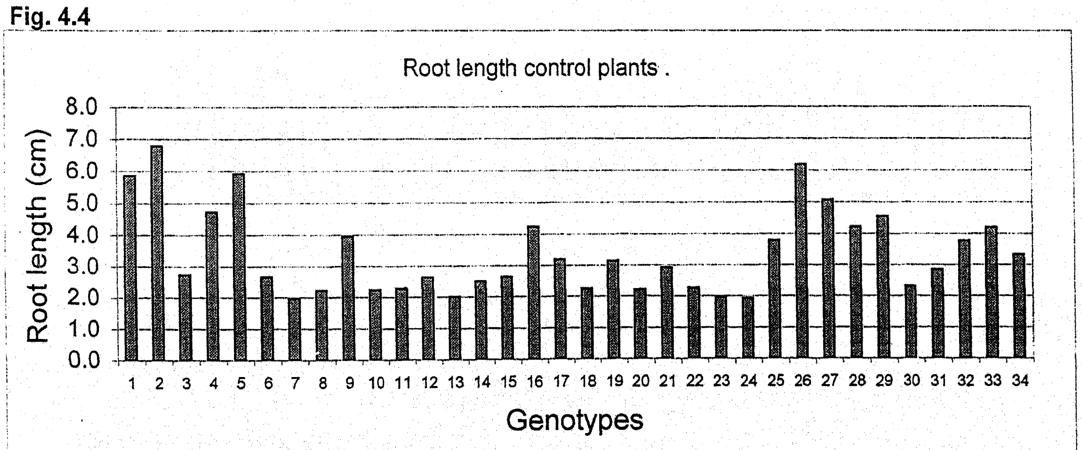


Fig. 4.4



1. EC 329299, **2**. EC 318954, **3**. Wardan, **4**. EC 407709, **5**. EC 400976, **6**. EC 508311, **7**. EC 4017103, **8**. EC 400977, **9**. EC 401711, **10**. ISH 34/49, **11**. ISH 34/41, **12**. ISH 34/11, **13**. Penta 99, **14**. Raj Bundi, **15**. Penta 99-1, **16**. ES 99, **17**. ISH 32/8/1, **18**. Wardan S2, **19**. ISH 26/50/7, 20. ISH 32/34/1, **21**. Multi-98-45, **22**. ISH 34/5/1, **23**. Raj 49/50, **24**. T 5-90I-1, **25**. T 45-1, **26**. T 44-4, **27**. ISH 8020B, **28**. ISH 8020Y, **29**. ISH 5050B, **30**. ISH 5050Y, **31**. ISH 34/8B, **32**. ISH 34/8Y, **33**. T 5-90-I, **34**. T-9-90FM.

Fig. 4.5

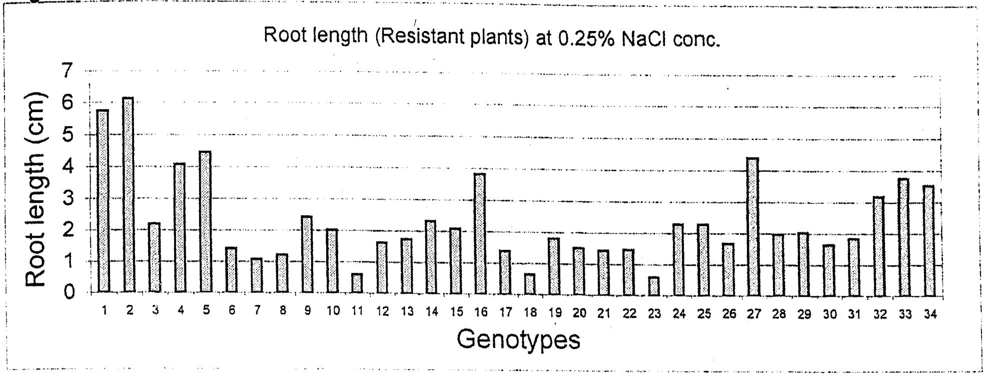


Fig. 4.6

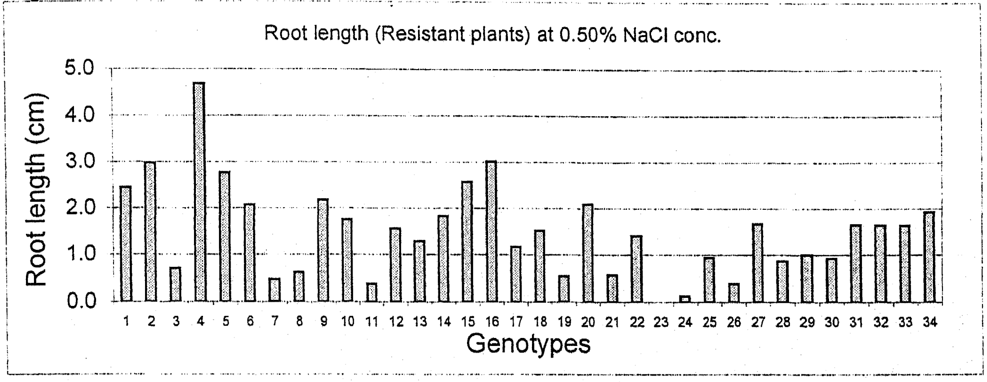


Fig. 4.7

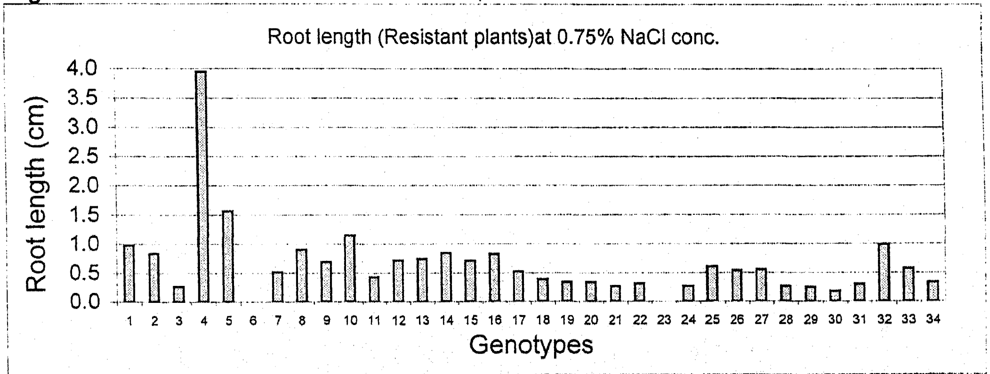
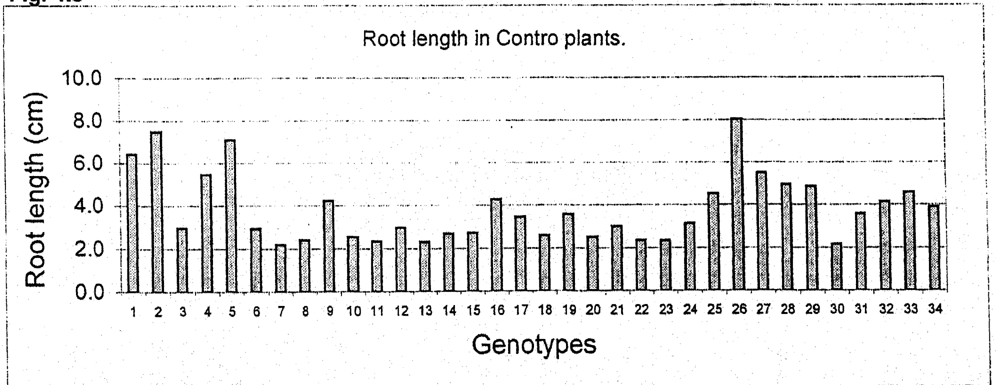


Fig. 4.8



1. EC 329299, **2**. EC 318954, **3**. Wardan, **4**. EC 407709, **5**. EC 400976, **6**. EC 508311, **7**. EC 4017103, **8**. EC 400977, **9**. EC 401711, **10**. ISH 34/49, **11**. ISH 34/41, **12**. ISH 34/11, **13**. Penta 99, **14**. Raj Bundi, **15**. Penta 99-1, **16**. ES 99, **17**. ISH 32/8/1, **18**. Wardan S2, **19**. ISH 26/50/7, 20. ISH 32/34/1, **21**. Multi-98-45, **22**. ISH 34/5/1, **23**. Raj 49/50, **24**. T 5-90I-1, **25**. T 45-1, **26**. T 44-4, **27**. ISH 8020B, **28**. ISH 8020Y, **29**. ISH 5050B, **30**. ISH 5050Y, **31**. ISH 34/8B, **32**. ISH 34/8Y, **33**. T 5-90-I, **34**. T-9-90FM.

Fig. 5.1

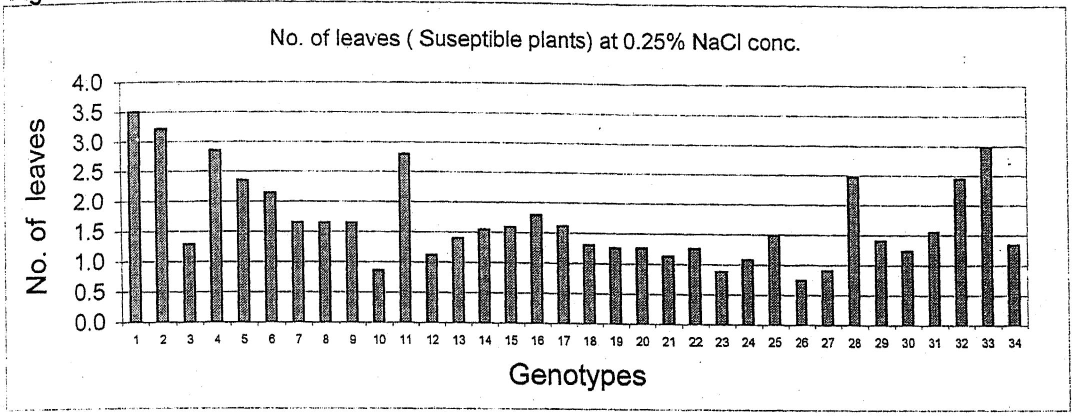


Fig. 5.2

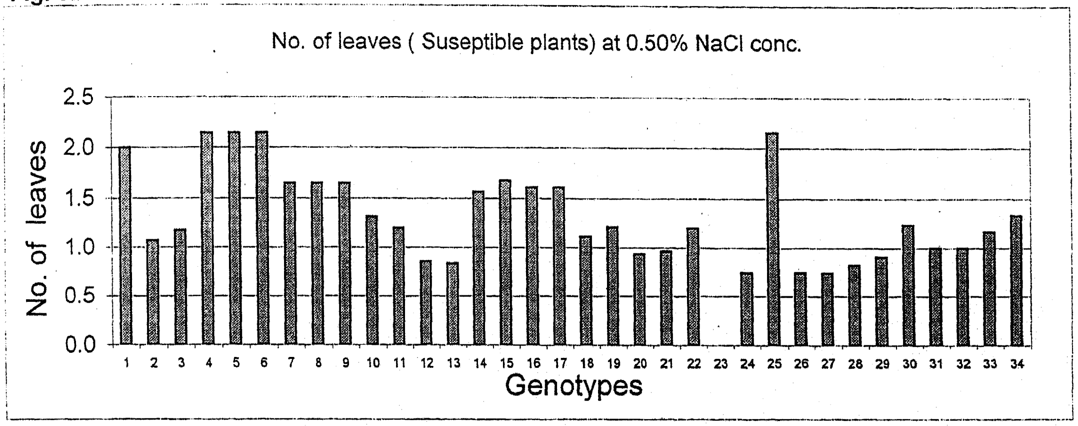


Fig. 5.3

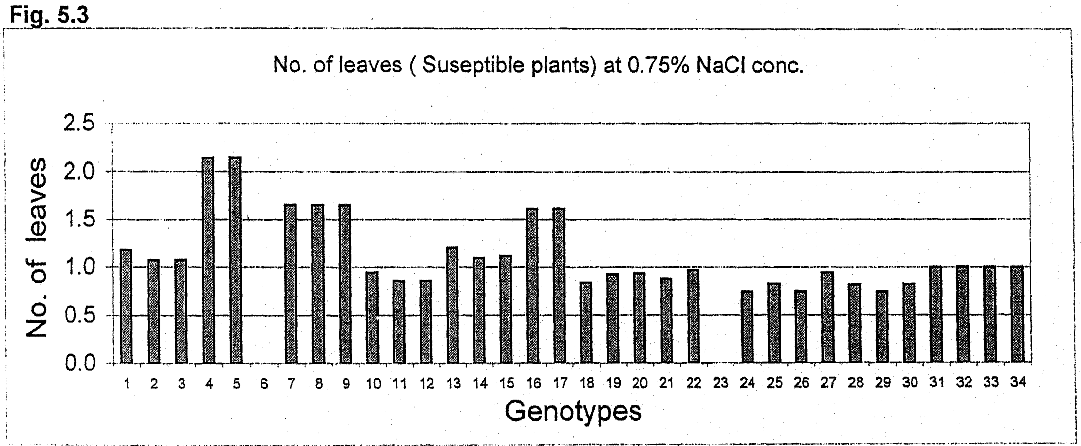
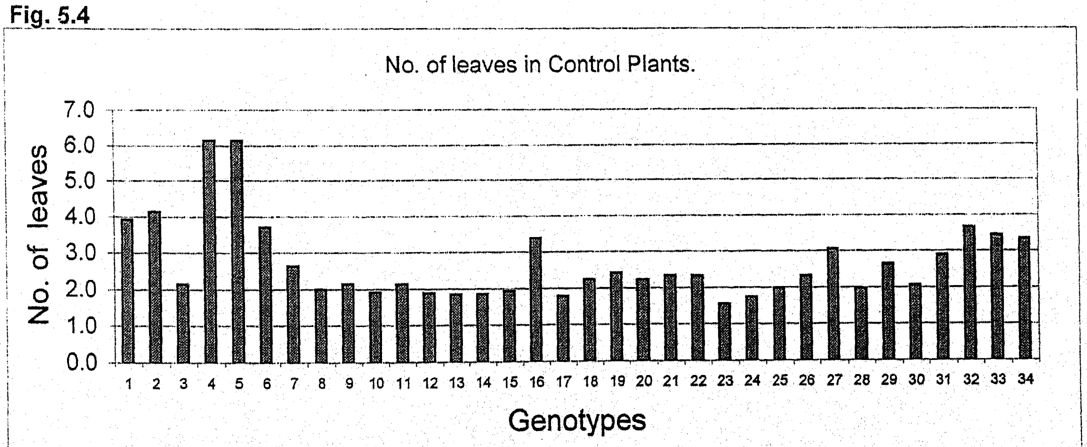


Fig. 5.4



1. EC 329299, **2**. EC 318954, 3. Wardan, 4. EC 407709, **5**. EC 400976, **6**. EC 508311, **7**. EC 4017103, **8**. EC 400977, **9**. EC 401711, **10**. ISH 34/49, **11**. ISH 34/41, **12**. ISH 34/11, **13**. Penta 99, **14**. Raj Bundi, **15**. Penta 99-1, **16**. ES 99, **17**. ISH 32/8/1, **18**. Wardan S2, **19**. ISH 26/50/7, 20. ISH 32/34/1, **21**. Multi-98-45, **22**. ISH 34/5/1, **23**. Raj 49/50, **24**. T 5-90I-1, **25**. T 45-1, **26**. T 44-4, **27**. ISH 8020B, **28**. ISH 8020Y, **29**. ISH 5050B, **30**. ISH 5050Y, **31**. ISH 34/8B, **32**. ISH 34/8Y, **33**. T 5-90-I, **34**. T-9-90FM.

Fig. 5.5

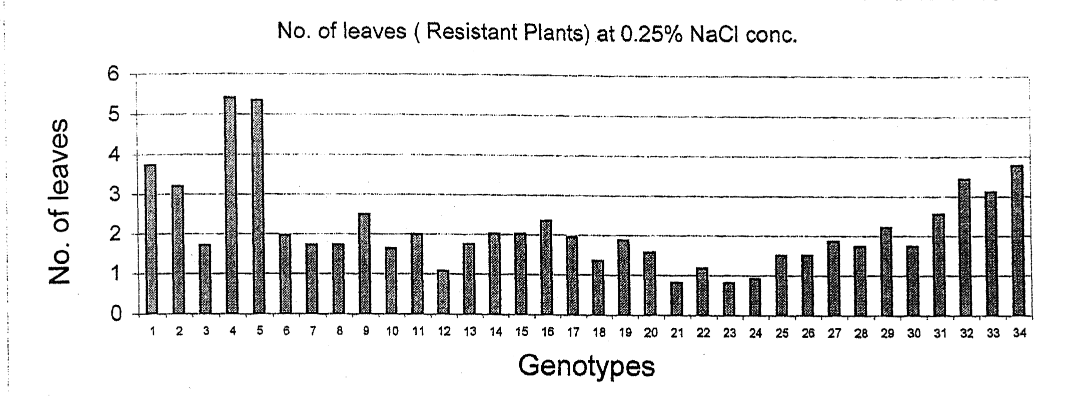


Fig. 5.6

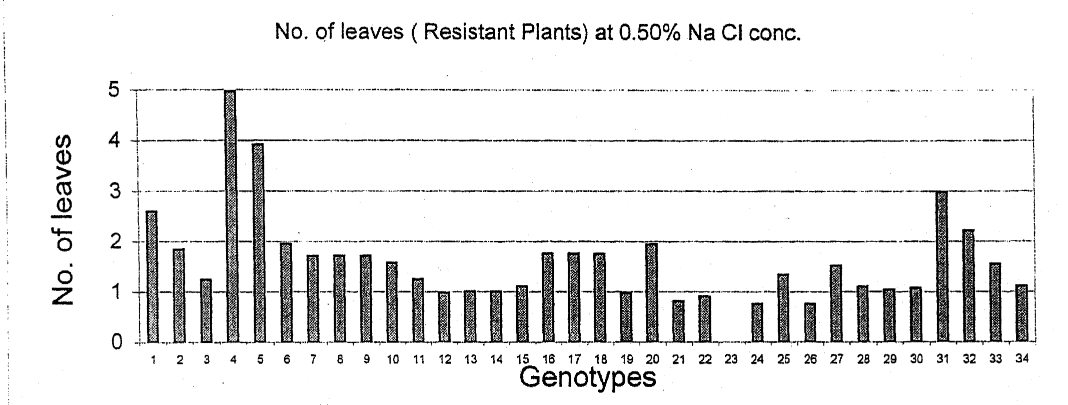


Fig. 5.7

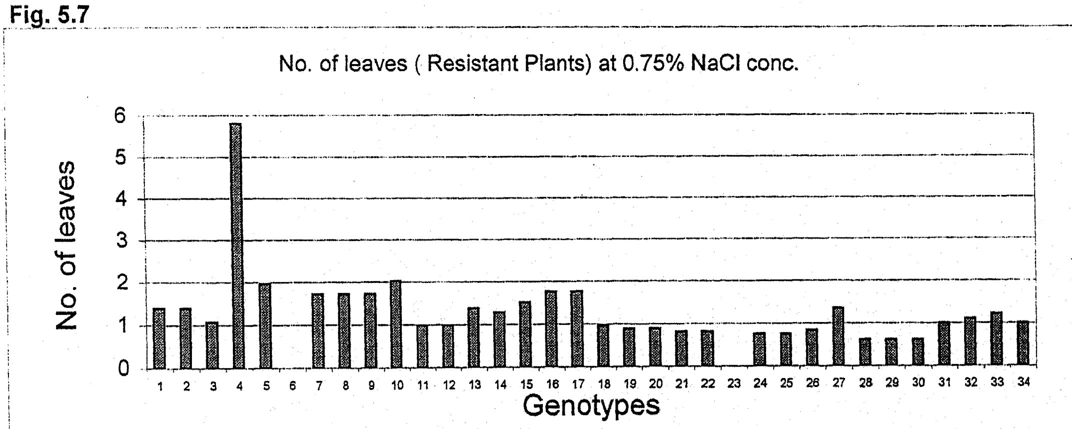
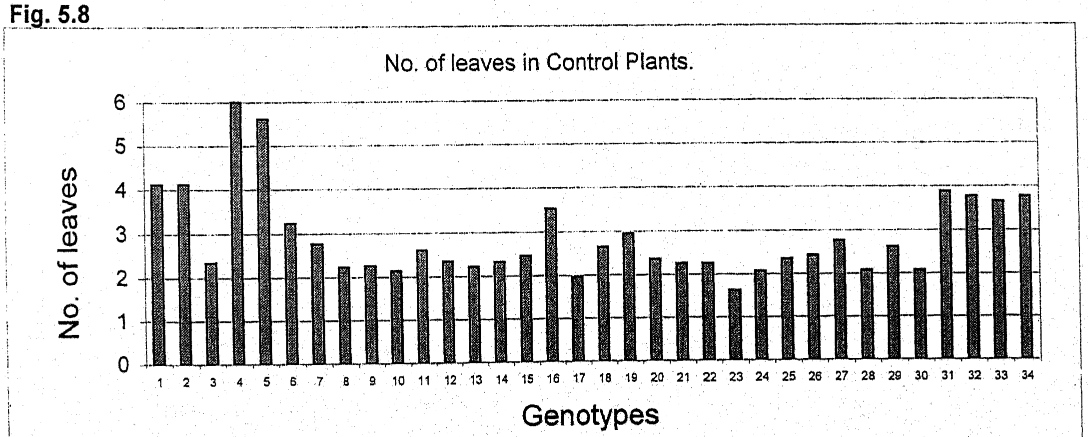


Fig. 5.8



1. EC 329299, **2.** EC 318954, **3.** Wardan, **4.** EC 407709, **5.** EC 400976, **6.** EC 508311, **7.** EC 4017103, **8.** EC 400977, 9. EC 401711, **10.** ISH 34/49, **11.** ISH 34/41, **12.** ISH 34/11, **13.** Penta 99, **14.** Raj Bundi, **15.** Penta 99-1, **16.** ES 99, **17.** ISH 32/8/1, **18.** Wardan S2, **19.** ISH 26/50/7, 20. ISH 32/34/1, **21.** Multi-98-45, **22.** ISH 34/5/1, **23.** Raj 49/50, **24.** T 5-90I-1, **25.** T 45-1, **26.** T 44-4, **27.** ISH 8020B, **28.** ISH 8020Y, **29.** ISH 5050B, **30.** ISH 5050Y, **31.** ISH 34/8B, **32.** ISH 34/8Y, **33.** T 5-90-I, **34.** T-9-90FM.

Fig. 6.1

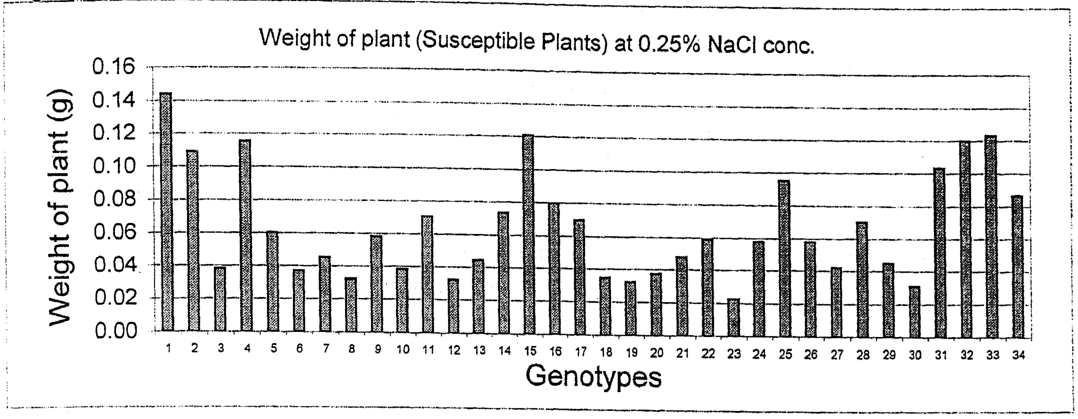


Fig. 6.3

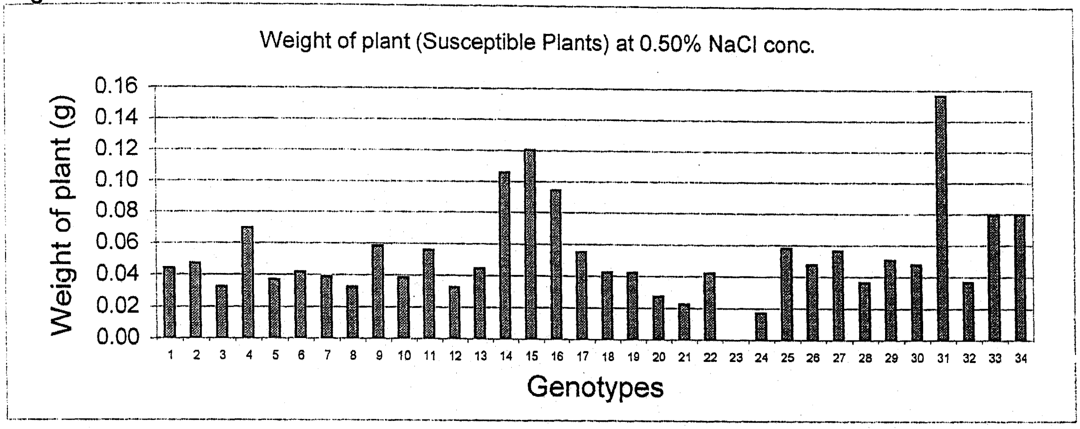


Fig. 6.5

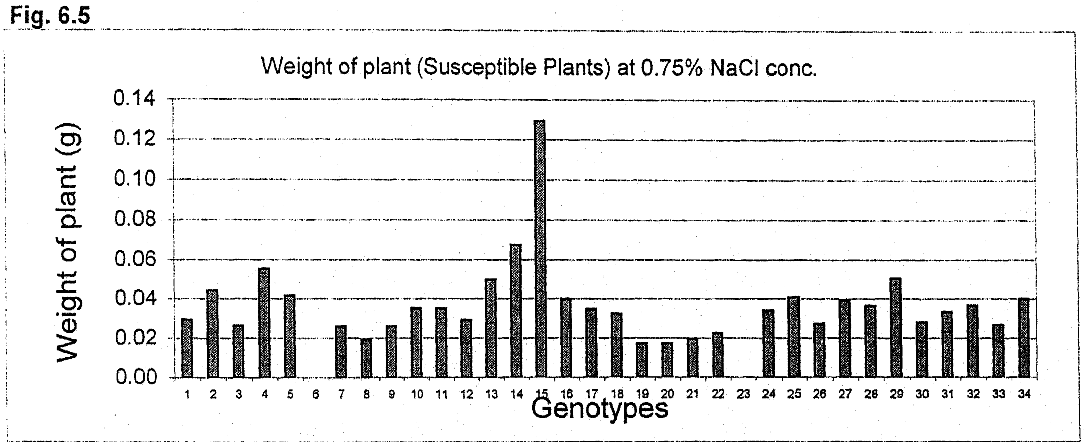
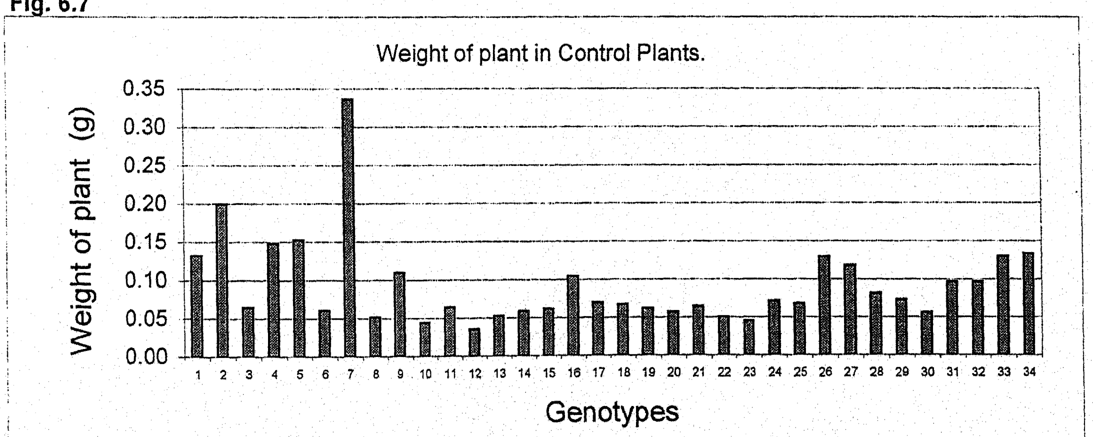


Fig. 6.7



1. EC 329299, **2.** EC 318954, **3.** Wardan, **4.** EC 407709, **5.** EC 400976, **6.** EC 508311, **7.** EC 4017103, **8.** EC 400977, **9.** EC 401711, **10.** ISH 34/49, **11.** ISH 34/41, **12.** ISH 34/11, **13.** Penta 99, **14.** Raj Bundi, **15.** Penta 99-1, **16.** ES 99, **17.** ISH 32/8/1, **18.** Wardan S2, **19.** ISH 26/50/7, 20. ISH 32/34/1, **21.** Multi-98-45, **22.** ISH 34/5/1, **23.** Raj 49/50, **24.** T 5-90I-1, **25.** T 45-1, **26.** T 44-4, **27.** ISH 8020B, **28.** ISH 8020Y, **29.** ISH 5050B, **30.** ISH 5050Y, **31.** ISH 34/8B, **32.** ISH 34/8Y, **33.** T 5-90-I, **34.** T-9-90FM.

Fig. 6.5

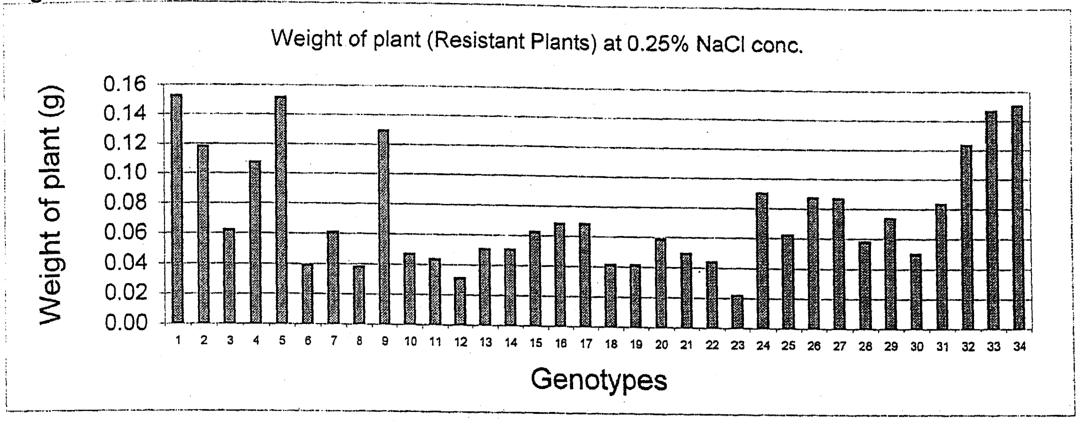


Fig. 6.6

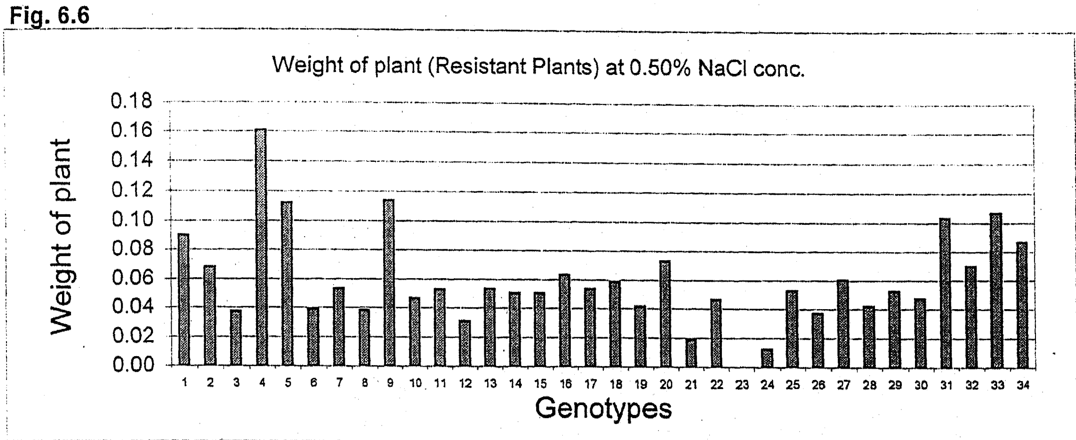


Fig. 6.7

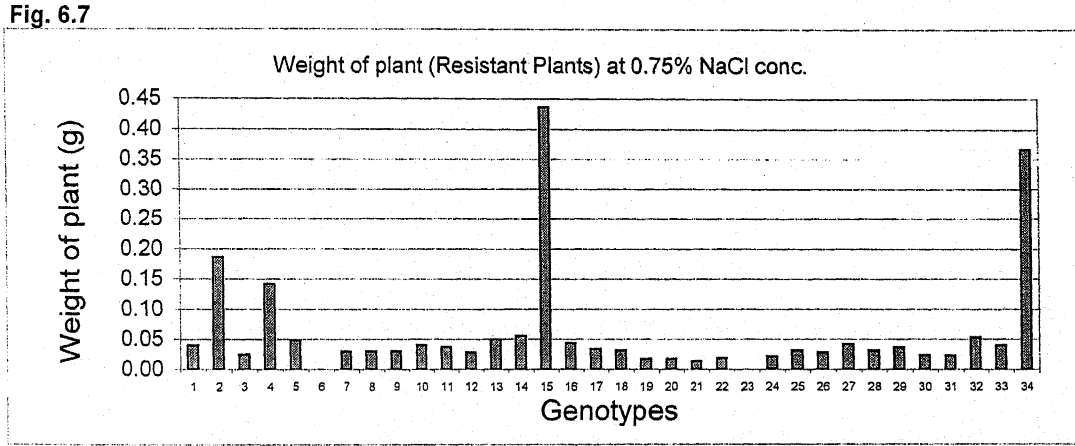
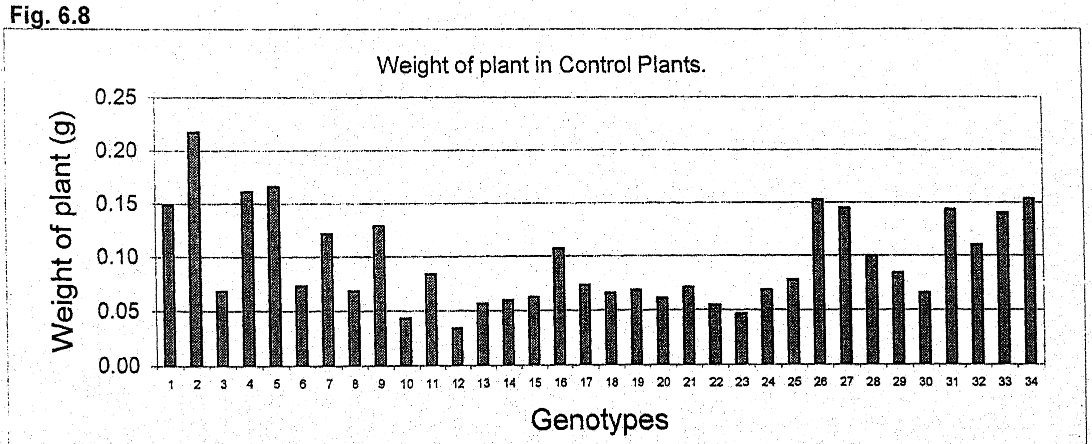


Fig. 6.8



1. EC 329299, **2**. EC 318954, **3**. Wardan, **4**. EC 407709, **5**. EC 400976, **6**. EC 508311, **7**. EC 4017103, **8**. EC 400977, **9**. EC 401711, **10**. ISH 34/49, **11**. ISH 34/41, **12**. ISH 34/11, **13**. Penta 99, **14**. Raj Bundi, **15**. Penta 99-1, **16**. ES 99, **17**. ISH 32/8/1, **18**. Wardan S2, **19**. ISH 26/50/7, 20. ISH 32/34/1, **21**. Multi-98-45, **22**. ISH 34/5/1, **23**. Raj 49/50, **24**. T 5-90I-1, **25**. T 45-1, **26**. T 44-4, **27**. ISH 8020B, **28**. ISH 8020Y, **29**. ISH 5050B, **30**. ISH 5050Y, **31**. ISH 34/8B, **32**. ISH 34/8Y, **33**. T 5-90-I, **34**. T-9-90FM.

Plant weight, resistant: The effect of genotypes, different levels of salinity treatments and their interaction for weight of plants in the resistant type was found to be highly significant.

Germination: The effect of genotypes, different levels of salinity and their interaction for germination percent was found to highly significant.

B. *In vitro* plant growth under saline vis-à-vis normal condition.

Data for all 34 genotypes was recorded for shoot length, root length, number of leaves, biomass of plant and mortality on 45th day of the plant growth under saline condition *in vitro* and presented in Table 11 and Fig 7 to 10. Data on total number of seeds germinated on 45th day is also presented because in some cases a few seeds germinated even after observation on 20th day.

EC 329299

Germination: The germination by 45th day in this genotype was 100%, 85%, 60% and 95% at 0.25%, 0.50%, 0.75% and control conditions. Thus, exposure to saline conditions did not reduce the germination at 0.25% and 0.50% salinity but at 0.75% salinity it was reduced. Prolonged exposure to saline conditions had deleterious affect on the plants 11.76% mortality was observed at 0.50% salinity whereas at 0.75% the process of degeneration was rapid and 75% of the seedlings had degenerated.

Shoot length: The average shoot length of the seedlings growing under control condition was 15.4 cm, which reduced to 8.8, 4.3 and 1.7 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average length of root in plants growing in control condition was 17.2, which marginally increased to 18.2 cm at 0.25% cm but at 0.50% and 0.75% it was substantially reduced to 4.7 and 1.8 cm.

Number of leaves: The average number of leaves in the plants growing under control condition was 7.0, which reduced to 5.3, 4.3 and 1.0 at 0.25%, 0.50% and 0.75% salinity.

Weight of plant: The average biomass production under control condition was 418 mg, which reduced to 191, 121 and 50 mg at 0.25%, 0.50% and 0.75% salinity.

EC 318954

Germination: Exposure to saline conditions had no affect on germination at 0.25% and 0.50% salinity and 100% seeds germinated whereas at 0.75% it was 80% and under control condition it was 100%. Prolonged exposure to saline condition had a deleterious

effect at higher salinity and 18.75% seedlings had completely degenerated by 45th day at 0.75% whereas 5% plants degenerated at 0.50% salinity and at 0.25% salinity no mortality was observed.

Shoot length: The average shoot length of the plants growing under control condition was 15.1 cm, which reduced to 10.3, 5.3 and 2.8 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 23.1 cm which reduced to 13.4 and 13.8 cm at 0.25% and 0.50% salinity respectively whereas at 0.75% it substantially reduced to 2.3 cm.

Number of leaves: The average number of leaves in the plants growing under control condition was 7.3, which reduced to 5.3, 5.0 and 2.0 at 0.25%, 0.50% and 0.75% salinity respectively. .

Weight of plant: The average biomass production of the plants growing under control condition was 476 mg which reduced to 199, 175 and 90 mg at 0.25%, 0.50% and 0.75% salinity; respectively.

Wardan

Germination: The germination by 45th days was 80, 70, 25 and 85% at 0.25%, 0.50%, 0.75% and control condition. Thus, exposure to saline condition had marginal affect on germination at 0.25% and 0.50% treatments, but higher saline treatment i.e., 0.75% substantially reduced the germination as compared to control. Prolonged exposure to saline condition resulted in the degeneration process in the plants and 18.75%, 35.7% and 40% plant mortality was observed at 0.25%, 0.50% and 0.75% salinity.

Shoot length: The average shoot length of the plants growing under control condition was 6.6 cm that reduced to 2.5, 2.4 and 1.1 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 14.3 cm, which reduced to 4.5, 1.8 and 0.6 cm at 0.25%, 0.50% and 0.75% salinity.

Number of leaves: The average number of leaves growing in control condition was 4.3, which reduced to 3.0, 3.3 and 1.5 respectively at 0.25 %, 0.50% and 0.75% respectively.

Weight of plant: The average biomass production of the control plants was 139 mg which was reduced to 76, 61 and 57 mg at 0.25%, 0.50% and 0.75% salinity respectively.

EC 407709

Germination: The total germination observed by 45th day was 85%, 80%, 70% at 0.25%, 0.50% and 0.75% salinity respectively whereas under control condition the

germination was 100%. Thus, increasing levels of salinity had marginal inhibitory affect on germination. Prolonged exposure to higher level of salinity initiated the degeneration process in the plants. About 6.25% plants at 0.50% salinity and 14.28% at 0.75% salinity had degenerated completely by 45th day however, no mortality was observed at 0.25% salinity.

Shoot length: The average shoot length of the plants growing under control condition was 8.1 cm which reduced to 3.1, 2.3 and 2.8 cm at 0.25%, 0.50% and 0.75% respectively.

Root length: The average root length of the plants growing under control condition was 18.0 cm, which reduced to 4.7, 1.8, 5.7 cm respectively at 0.25%, 0.50% and 0.75% salinity level respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 4.5 which reduced to 2.0, 2.3 and 3.0 at 0.25%, 0.50% and 0.75% salinity level respectively.

Weight of plant: The average biomass production of the plants growing under control condition was 203 mg that was reduced to 89, 82 and 99mg at 0.25%, 0.50% and 0.75% salinity.

EC 4017103

Germination: Saline conditions greatly inhibited the germination in this genotype. Germination under control condition was 80%, which was reduced to 35, 25 and 20% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline conditions had a deleterious effect on the plants. By 45th days 14.28, 40 and 100% seedlings degenerated in the 0.25%, 0.50% and 0.75% salinity treatments.

Shoot length: The average shoot length of the plants growing under control condition was 10.1cm, which reduced to 2.1 and 1.8 cm at 0.25% and 0.50% salinity whereas at 0.75% salinity no plant survived.

Root length: The average root length of the plants growing under control condition was 18.5 cm, which was highly inhibited to 1.0 cm and 0.5 cm at 0.25% and 0.50% salinity levels.

Number of leaves: - The average number of leaves in the plants growing under control condition was 5.0 which reduced to 1.3 and 1.0 at 0.25% and 0.50% salinity respectively.

Weight of plant: The average biomass production in the plants growing under control condition was 266 mg, which reduced to 36 and 44 mg at 0.25% and 0.50 % salinity.

EC 400977

Germination: Saline conditions inhibited the germination in this genotype. Germination under control condition was 95%, which reduced to 45, 15 and 10% at 0.25%, 0.50% and 0.75% salinity respectively. Salinity inhibited the growth of seedlings to a great extent at 0.25% and 0.50% but no mortality was observed, however, at 0.75% salinity no plant survived.

Shoot length: The average shoot length of the plants growing under control condition was 13.9 cm that reduced to 1.2 and 0.8 cm at 0.25% and 0.50% salinity.

Root length: The average root length of the plants growing under control condition was 20.1 cm whereas at 0.25% and 0.50% salinity there was no root development.

Number of leaves: The average number of leaves in the plants growing under control condition was 7.0 whereas the development of leaves was completely inhibited under salinity treatment 0.25%, 0.50% and 0.75% salinity and the plants degenerated by 45th day.

Weight of plants: The average weight of plants growing under control condition was 449 mg, which was reduced to 250 mg at 0.25% and at 0.50% it was significantly reduced to 48 mg.

EC 400976

Germination: The overall germination was least affected due to salt –stress condition at 0.25% and 0.50% salinity levels but at 0.75% salinity it was drastically reduced. The germination was 100, 95 and 16% at 0.25%, 0.50% and 0.75% salinity respectively whereas under control condition it was 90%. Prolonged exposure to saline conditions particularly at 0.75% salinity had a high deleterious effect on the plants and all the plants degenerated by 45th day. However, at 0.25% and 0.50% salinity no mortality was observed.

Shoot length*:* The average shoot length of the plants growing under control condition was 8.3 cm, which reduced to 3.1 cm and 1.8 cm at 0.25% and 0.50% salinity respectively.

Root length: The average root length of the plants growing under control condition was 8.2 cm, which reduced to 1.8 and 1.2 cm at 0.25% and 0.50% salinity respectively.

Number of leaves: The average number leaves in the plants growing under control condition were 5.0, which reduced to 2.8 and 2.0 cm at 0.25% and 0.50% salinity respectively.

Weight of plant: The average biomass production in the plants growing under control condition was 207 mg, which reduced to 107 and 59 mg at 0.25% and 0.50% salinity.

EC 508311

Germination: The rate of germination by 45th day was 80, 20 and 20% at 0.25%, 0.50% and 0.75% salinity whereas under control condition it was 100%. Thus, germination was reduced with increasing level of salinity. Prolonged exposure to salinity had deleterious affect on the plants and by 45th day 6.25% plants had degenerated at 0.25% salinity, whereas at 0.50% and 0.75% salinity 100% mortality was observed.

Shoot length: The average shoot length of the plants growing under control condition was 6.1 cm that reduced to 2.1 cm at 0.25% salinity.

Root length: The average root length of the plants growing under control condition was 10.0 cm that reduced to 1.4 cm at 0.25% salinity.

Number of leaves: The average number of leaves in the plants growing under control condition was 4.0, which reduced to 1.6 at 0.25%.

Weight of plant: The average biomass production in the plants growing under control condition was 154 mg that reduced to 51 mg at 0.25% salinity.

EC 401711

Germination: Germination under control condition was 100%, which reduced under increasing salinity. The germination was 50, 25 and 25% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline condition had deleterious effect; all the plants at 0.50 and 0.75% degenerated whereas at 0.25% salinity 11.1% plants degenerated.

Shoot length: The average shoot length of the plants growing under control condition was 11.1 cm that reduced substantially to 2.9 cm at 0.25% salinity.

Root length: The average root length of the plants growing under control condition was 13.5 cm, which reduced to 5.7 cm at 0.25%.

Number of leaves: The average number of leaves in the plants growing under control condition was 6.0, which reduced to 4.0 at 0.25% salinity.

Weight of plants: The average biomass production in the plants growing under control condition was 260 mg, which reduced significantly to 74 mg at 0.25%.

ISH 34/49

Germination: Germination under control condition was 100%, which gradually reduced as the level of salinity increased. The germination at 0.25%, 0.50% and 0.75% salinity was 90%, 70% and 40% respectively. Prolonged exposure to saline conditions

particularly higher salinity had deleterious effect on the plants and the degeneration process initiated. At 0.50% salinity 21.42% plants degenerated and in 0.75% salinity the morality was 100%. However, no mortality was observed at 0.25% salinity.

Shoot length: The average shoot length of the plants growing under control condition was 6.6 cm that reduced to 5.3 and 3.3 cm at 0.25% and 0.50% salinity respectively.

Root length: The average root length of the plants growing under control condition was 8.7 cm that reduced to 2.5 and 1.5 cm at 0.25 % and 0.50% salinity.

Number of leaves: The average number of leaves in the plants growing under control condition was 53, which reduced to 4.3 and 1.8 at 0.25% and 0.50% salinity.

Weight of plant: The average biomass production in the plants growing under control condition was 123 mg that reduced to 109 and 86 mg at 0.25% and 0.50% salinity.

ISH 34/41

Germination: The rate of germination under control condition was 95%, which was marginally reduced to 90, 80 and 80% at 0.25%, 0.50% and 0.75% salinity. Prolonged exposure to salinity initiated the degeneration process and 5.5%, 18.75% and 18.75% plants had degenerated by 45th day at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 7.9 cm, which reduced to 4.4, 2.6 and 1.9 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 15.6 cm, which reduced to 3.3 and 0.9 cm at 0.25% and 0.50% salinity whereas at 0.75% salinity the plants had no roots at all.

Number of leaves: The average number of leaves in the plant growing under control condition was 5.3 cm that was reduced to 3.8, 2.3 and 2.0 at 0.25%, 0.50% and 0.75 % salinity respectively.

Weight of plants: The average biomass production of the seedlings under control condition was 148 mg whereas it was 106 mg and 96mg at 0.25% and 0.50% salinity respectively

ISH 34/11

Germination: The germination under control condition was 100% while it was 90, 70 and 60% at 0.25%, 0.50% and 0.75% salinity. Thus, as the level of salinity was increased the germination decreased. Prolonged exposure to 0.75% salinity initiated the degeneration process in the plants and by 45th day 6.25% plants degenerated whereas no mortality was observed at 0.25% and 0.50% salinity.

Shoot length: The average shoot length of the plants growing under control condition was 6.0 cm that reduced to 3.6, 2.0 and 1.1 at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 10.8 cm, which was reduced to 2.6 and 1.0 cm at 0.25% and 0.50% salinity respectively whereas at 0.75% the plants had no root development.

Number of leaves: The average number leaves in the plants growing under control condition were 3.7 which reduced to 2.3, 1.6 and 1.0 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plant: - The average biomass production in the plants growing under control condition was 119 mg which reduced to 53, 43 and 39 mg at 0.25%, 0.50% and 0.75% salinity respectively.

Penta 99

Germination: The germination under control condition was 75%, which was reduced to 55, 60 and 60% at 0.25%, 0.50% and 0.75% salinity respectively. Thus, increasing levels of salinity marginally reduced the germination rate. Prolonged exposure to saline condition had deleterious effect on the plants and the mortality rate was 18, 33 and 100% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average length of the shoot in the plants growing under control condition was 5.2 cm, which reduced to 3.8 and 2.2 cm at 0.25% and 0.50% salinity respectively.

Root length: The average length of the root in the plants growing under control condition was 4.9 cm, which reduced to 2.2 and 1.0 cm at 0.25% and 0.50% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.3 which reduced to 2.5 and 1.3 cm at 0.25% and 0.50 salinity.

Weight of plant: The average biomass production in the plants growing under control condition was 120 mg, which reduced to 91 mg and 80 mg at 0.25% and 0.50% salinity level respectively.

Raj Bundi

Germination: Germination under control condition was 75% whereas it was 80, 55 and 50% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline condition had deleterious effect on the plants and mortality rate was 6.25, 27.2 and 100% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 5.6 cm, which reduced to 3.9 and 2.2 cm at 0.25% and 0.50% salinity level.

Root length: The average root length of the plants growing under control condition was 7.5 cm, which reduced to 2.1 and 1.5 cm at 0.25% and 0.50% salinity level respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.3, which reduced to 2.7 and 2.2 at 0.25% and 0.50% salinity level respectively.

Weight of plants: The average biomass production in the plants growing under control condition was 125 mg which reduced to 91 and 80 mg at 0.25% and 0.50% salinity respectively.

Penta 99-1

Germination: The germination of seeds under control condition was 85%, which was reduced to 80, 55 and 60% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to salinity conditions had a very deleterious effect on the plants at higher salinity i.e., 0.50% and 0.75%. The mortality rate was 12.5, 100 and 100% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 3.9 cm, which was reduced to 2.3 cm at 0.25% salinity.

Root length: The average root length of the plants growing under control condition was 2.9 cm, which was reduced to 1.8 cm at 0.25% salinity.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.0, which was reduced to 1.7 at 0.25% salinity.

Weight of plant: The average biomass production in the plants growing under control condition was 98 mg, which was same at 0.25% salinity.

ES 99

Germination: The seed germination under control condition was 85% which was reduced to 70, 55 and 50% at 0.25%, 0.50% and 0.75% salinity level respectively. 30% of the plants degenerated by 45^{th} day at 0.25% salinity whereas at 0.50% and 0.75% salinity no plant survived.

Shoot length: The average shoot length of the plants growing under control condition was 5.6 cm which was reduced to 2.9 cm at 0.25% salinity.

Root length: The average root length of the plants growing under control condition was 7.6 cm, which reduced to 1.9 cm at 0.25% salinity.

Number of leaves: The average number of leaves of the plants growing under control condition was 3.3, which reduced to 1.5 at 0.25% salinity.

Weight of plant: The average biomass of the plants growing under control condition was 139 mg, which reduced to 70 mg at 0.25% salinity.

ISH 32/8/1

Germination: The overall germination under control condition was 85%, which reduced to 50, 55 and 25% at 0.25%, 0.50% and 0.75% salinity respectively. Thus, a gradual decrease in the germination of seeds was observed as the level of salinity was increased. Prolonged exposure to saline conditions had a deleterious effect on the plants and the plants had started degenerating. The mortality rate was 30, 45.5 and 100% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 3.8 cm, which reduced to 2.5 and 1.6 cm at 0.25% and 0.50% salinity respectively.

Root length: - The average root length of the plants growing under control condition was 5.1 cm which reduced to 1.0 and 0.9 cm at 0.25% and 0.50% salinity.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.0 whereas at 0.25% and 0.50% salinity it was 1.0 and 1.0 respectively.

Weight of plant: The average biomass production in the plants growing under control condition was 101 mg, which reduced to 58 and 53 mg.

Wardan S2

Germination: The overall germination by 45th day under control condition was 100%, which was reduced to 85, 55 and 30% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline condition started the degeneration process particularly at 0.50% and 0.75% salinity. The rate of mortality was 11.76, 90.09 and 100% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 7.6 cm, which reduced to 4.5 and 1.0 cm at 0.25% and 0.50% salinity.

Root length: The average root length of the plants growing under control condition was 2.2 cm, which was inhibited under salinity conditions to 1.0 and 0.5 cm at 0.25% and 0.50% salinity.

Number of leaves: The average number of leaves in the plants growing under control condition was 5.0, which reduced to 3.3 and 1.0 at 0.25% and 0.50% salinity.

Weight of plant: The average biomass production in the plants growing under control condition was 145 mg, which reduced to 127 mg and 23 mg at 0.25% and 0.50% salinity.

ISH 26/50/7

Germination: The overall germination under control condition by 45th day was 100%, which reduced to 75, 75 and 40% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline conditions had started the degeneration process in the plants. The mortality rate was 26.66, 26.66 and 50% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 7.9 cm, which was reduced to 3.0, 2.1 and 1.0 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 3.4 cm, which was reduced to 1.0 cm at 0.25% salinity whereas at 0.50% and 0.75% salinity the growth of roots was completely inhibited.

Number of leaves: The average number of leaves in the plants growing under control condition was 5.7, which reduced to 2.5 and 1.3 at 0.25% and 0.75% whereas at 0.75% the development of leaves was inhibited.

Weight of plants: The average biomass production in the plants growing under control condition was 155 mg, which was reduced to 103, 71 and 19 mg at 0.25%, 0.50% and 0.75% salinity respectively.

ISH 32/34/1

Germination: Overall germination under control condition was 90%, whereas at 0.25% salinity 95% of the seeds germinated whereas at 0.50% and 0.75% salinity it was 75 and 30% respectively. Prolonged exposure to saline condition initiated the degeneration process in the plants. The mortality rate was 10.52, 13.33 and 90% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 4.8 cm, which was gradually reduced to 4.1, 2.0 and 1.5 at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 4.5 cm, which reduced to 3.5, 1.1 and 0.4 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.7, which reduced to 3.5 and 1.6 at 0.25% and 0.50% salinity respectively whereas at 0.75% the growth of leaves was inhibited completely.

Weight of plant: The average biomass production in the plants growing under control condition was 91 mg that marginally increased to 98 mg at 0.25% whereas at 0.50% and 0.75% it was 61 mg and 48 mg respectively.

Multi-98-45

Germination: The overall germination under control condition was 85%, which was reduced to 45, 45 and 20% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure of plants to saline condition particularly higher salinity level had a very deleterious effect on plants. At 0.75% salinity the mortality rate was 100% but at 0.25% and 0.50% salinity there was no mortality.

Shoot length: The average shoot length of the plants growing under control condition was 6.4 cm, which reduced to 2.7 and 1.6 cm at 0.25% and 0.50% salinity respectively.

Root length: The average root length of the plants growing under control condition was 3.0 cm, which reduced to 1.3 and 0.7 cm at 0.25% and 0.50% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 4.0, which reduced to 2.8 and 2.0 at 0.25% and 0.50% salinity respectively.

Weight of plant: The average biomass production in the plants growing under control condition was 138 mg, which reduced to 90 and 74 mg at 0.25% and 0.50% salinity respectively.

ISH 34/5/1

Germination: The overall germination under control condition was 90%, which reduced to 75, 60 and 20% at 0.25%, 0.50% and 0.75% salinity respectively. The prolonged exposure of plants to increasing levels of salinity had a deleterious effect on the plants and by 45th day 13.3, 41.66 and 100% plants degenerated at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 6.0 cm, which reduced to 2.8 and 1.5 at 0.25% and 0.50% salinity respectively.

Root length: The average root length of the plants growing under control condition was 3.2 cm, which reduced to 0.9 and 0.9 cm at 0.25% and 0.50 % salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 4.0 that reduced to 2.8 and 2.0 at 0.25% and 0.50% salinity respectively.

Weight of plant: The average biomass production in the plants growing under control condition was 108 mg, which reduced to 105 and 84 mg at 0.25% and 0.50% salinity respectively.

Raj 49/50

Germination: The overall germination under control condition was 65%, which was highly inhibited at 0.25% to 10%, and at 0.50% and 0.75% salinity there was no germination. Thus, this genotype was very sensitive to saline conditions as far as the germination process was concerned. Saline condition had a high deleterious effect on the plants and 100% mortality was observed at 0.25% salinity whereas at 0.50% and 0.75% no germination was observed.

Shoot length: The average shoot length of the plants growing under control condition was 4.9 cm whereas the plants at 0.25% had completely degenerated by 45th day and no germination was reported at 0.50% and 0.75% salinity

Root length: The average root length of the plants growing under control condition was 2.8 cm.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.0.

T 44-4

Germination: The overall germination under control condition was 100%, which reduced to 75, 50 and 60% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline conditions had initiated the degeneration process in the plants. The rate of mortality in the plants was 20, 40 and 83.33% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 8.1 cm, which reduced to 3.1, 0.5 and 0.6 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 14.1, which inhibited to 1.5 cm at 0.25% salinity whereas the plants growing at 0.50% and 0.75% salinity had no roots at all.

Number of leaves: The average number of leaves in the plants growing under control condition was 5.0, which reduced to 2.5 at 0.25% salinity whereas at 0.50% and 0.75% the plants remained at cotyledonary leaf stage.

Weight of plants: The average biomass production in the plants growing under control condition was 240 mg, which was reduced to 79, 17 and 19 mg at 0.25%, 0.50% and 0.75% salinity respectively.

T 45-1

Germination: The overall germination under control condition was 90%, which gradually reduced to 75, 60 and 50% at 0.25%, 0.50% and 0.75% salinity level respectively. Higher level of salinity i.e., 0.75% had a deleterious effect on the plants and 30% mortality was observed, whereas at 0.25% and 0.50% salinity no mortality was observed.

Shoot length: The average shoot length of the plants growing under control condition was 10.0 cm, which reduced 2.4, 2.4 and 2.1 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 14.0 cm, which reduced to 1.4, 1.8 and 0.8 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 5.3, which reduced to 2.0, 3.0 and 3.0 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plants: The average biomass production in the plants growing under control condition was 181 mg, which was reduced to 65, 101 and 93 mg at 0.25%, 0.50% and 0.75% salinity respectively. Thus, average biomass of the plants at higher salinity i.e., 0.50% and 0.75% was more than at low salinity i.e., 0.25% salinity.

T 5-90I-1

Germination: The overall germination under control condition was 100%, which was reduced to 85, 25 and 20% at 0.25%, 0.50% and 0.75% salinity level. Thus, higher salinity level greatly inhibited the germination process. The mortality rate was 0, 40 and 75% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 4.1 cm, which reduced to 3.4, 1.8 and 1.2 cm at 0.25%, 0.50% and 0.75% salinity level respectively.

Root length: The average root length of the plants growing under control condition was 3.7 cm, which reduced to 1.8, 0.4 and 0.6 cm at 0.25%, 0.50% and 0.75% salinity level respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.7, which reduced to 3.0, 2.0 and 1.0 at 0.25%, 0.50% and 0.75% salinity level respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.6, which reduced to 2.3 at 0.25% salinity condition whereas the plants growing at 0.50% salinity were at cotyledonary leaf stage.

Weight of plant: The average biomass of the seedlings growing under control condition was 180 mg which reduced to 88 and 42 mg at 0.25% and 0.50% salinity respectively.

ISH 5050B

Germination: The overall seed germination under control condition was 85% which reduced to 65, 40 and 55% at 0.25%, 0.50% and 0.75% salinity respectively. Higher levels of salinity initiated the degeneration process in this genotype and 25% and 36.36% plants degenerated completely at 0.50% and 0.75% salinity treatments whereas at 0.25% no mortality was observed.

Shoot length: The average shoot length of the plants growing under control condition was 5.9 cm, which reduced to 3.8, 1.7 and 2.4 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 8.3 cm, which reduced to 2.2, 0.9 and 1.1 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 4.3, which reduced to 3.2, 2.0 and 1.5 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plants: The average biomass production of the plants growing under control condition was 121 mg which reduced to 103, 63 and 90 mg at 0.25%, 0.50% and 0.75% salinity respectively.

ISH 5050Y

Germination: The overall seed germination under control condition was 85% which reduced to 75, 45 and 30% at 0.25%, 0.50% and 0.75% salinity level.

Shoot length: The average shoot length of the plants growing under control condition was 4.0 cm, which was reduced to 3.9 cm at 0.25%.

Root length: The average root length of the plants growing under control condition was 5.4 cm, which was reduced to 2.4 cm at 0.25%.

Number of leaves: The average number of leaves in the plants growing under control condition was 3.3, which reduced to 2.7 at 0.25% salinity level.

Weight of plants: The average biomass production in the plants growing under control condition was 111 mg whereas at 0.25% salinity it reduced to 86 mg.

Prolonged exposure to saline conditions had a very deleterious affect on the plants particularly at higher salinity level. At 0.25% salinity, 13.3% plants degenerated completely whereas at 0.50% and 0.75% salinity no plant survived till 45th day.

ISH 34/8B

Germination: The overall seed germination under control condition was 80% which was reduced to 65, 35 and 30% at 0.25%, 0.50% and 0.75% salinity level. Prolonged exposure to saline conditions initiated the degeneration process in the plants. The mortality rate was 15.3, 28.5 and 50% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 10.7 cm, which reduced to 5.5, 2.7 and 1.6 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 10.0 cm, which was reduced to 3.4, 1.4 and 0.5 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 5.7, which reduced to 4.2, 2.2 and 1.0 at 0.25%, 0.50% and 0.75% salinity level respectively.

Weight of plant: The average biomass of the plants growing under control condition was 215 mg whereas at 0.25% salinity it marginally increased to 220 mg but at 0.50% and 0.75% salinity it was substantially reduced to 94 and 64 mg respectively.

ISH 34/8Y

Germination: The overall seed germination under control condition was 100%, which reduced to 60, 60 and 75% at 0.25%, 0.50% and 0.75% salinity level respectively. Thus, the germination at 0.75% salinity was more than at 0.25% and 0.50% salinity. Prolonged exposure to saline condition particularly higher levels of salinity initiated the degeneration process in the plants, 25 and 20% mortality was observed at 0.50% and 0.75% salinity respectively. No mortality was observed at 0.25% salinity.

Shoot length: The average shoot length of the plants growing under control condition was 8.9 cm, which reduced to 4.8, 2.0 and 2.0 cm at 0.25%, 0.50% and 0.75% salinity level respectively.

Root length: The average root length of the plants growing under control was 9.9 cm, which was reduced to 5.2, 1.2 and 0.8 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 6.3 that reduced to 4.5, 1.5 and 2.0 at 0.25%, 0.50% and 0.75% salinity level respectively.

Weight of plants: The average biomass of the plants growing under control condition was 282 mg, which reduced to 207, 102 and 71 mg at 0.25%, 0.50% and 0.75% salinity respectively.

T 5-90-I

Germination: The overall germination of seeds under control condition was 85%, which reduced to 70, 55 and 55% at 0.25%, 0.50% and 0.75% salinity respectively. Prolonged exposure to saline condition initiated the degeneration process in the plants particularly at 0.50% and 0.75% wherein by 45th day 9.09% and 13.3% of the plants degenerated but low salinity treatment i.e., 0.25% had no mortality.

Shoot length: The average shoot length of the plants growing under control condition was 7.6 cm, which reduced to 5.6, 4.1 and 1.8 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Root length: The average root length of the plants growing under control condition was 10.1 cm, which was reduced to 4.2, 1.8 and 1.1 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 5.7, which was reduced to 3.7, 4.2 and 1.5 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plant: The biomass production of the seedlings under control condition was 217 mg whereas at 0.25% and 0.50% it was increased to 229 and 345 mg respectively whereas at 0.75% salinity it substantially reduced to 77 mg.

T-9-90FM

Germination: The overall germination of seeds under control condition was 100% whereas at 0.25%, 0.50% and 0.75% salinity it was 80, 55 and 65% respectively. Prolonged exposure to saline conditions initiated the degeneration process in the plants. The mortality rate was 6.25%, 27.27% and 22.2% at 0.25%, 0.50% and 0.75% salinity respectively.

Shoot length: The average shoot length of the plants growing under control condition was 7.7 cm, which reduced to 4.0, 3.3 and 1.4 cm at 0.25%, 0.50% and 0.75% salinity respectively.

Table 11. Germination, shoot length, root length and weight of 45 days old seelding of Egyptian clover genotypes growing *in vitro*

SN	Genotype	Shoot Length (cm)				Root Length (cm)				No. of Leaves				Weight of plant (g)			
		0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control	0.25%	0.50%	0.75%	Control
1	EC 329299	8.8	4.3	1.7	15.4	18.2	4.7	1.8	17.2	5.3	4.3	1.0	7.0	0.191	0.121	0.050	0.418
2	EC 318954	10.3	5.3	2.8	15.1	13.4	13.8	2.3	23.1	5.3	5.0	2.0	7.3	0.199	0.175	0.090	0.476
3	Wardan	2.5	2.4	1.1	6.6	4.5	1.8	0.6	14.3	3.0	3.3	1.5	4.3	0.076	0.061	0.057	0.139
4	EC 407709	3.1	2.3	2.8	8.1	4.7	1.8	5.7	18.0	2.0	2.3	3.0	4.5	0.089	0.082	0.099	0.203
5	EC 400976	3.1	1.8	NS	8.3	1.8	1.2	NS	8.2	2.8	2.0	NS	5.0	0.107	0.059	NS	0.207
6	EC 508311	2.1	NS	NS	6.1	1.4	NS	NS	10.0	1.6	NS	NS	4.0	0.051	NS	0.000	0.154
7	EC 4017103	2.1	1.8	NS	10.1	1.0	0.5	NS	18.5	1.3	1.0	NS	5.0	0.036	0.044	NS	0.266
8	EC 400977	1.2	0.8	NS	13.9	NR	NR	NR	20.1	NL	NL	NL	7.0	0.250	0.048	NS	0.449
9	EC 401711	2.9	NS	NS	11.1	5.7	NS	NS	13.5	4.0	NS	NS	6.0	0.074	NS	NS	0.260
10	ISH 34/49	5.3	3.3	NS	6.6	2.5	1.5	NS	8.7	4.3	1.8	NS	5.3	0.109	0.086	NS	0.123
11	ISH 34/41	4.4	2.6	1.9	7.9	3.3	0.9	NR	15.6	3.8	2.3	2.0	5.3	0.106	0.096	0.087	0.148
12	ISH 34/11	3.6	2.0	1.1	6.0	2.6	1.0	NR	10.8	2.3	1.6	1.0	3.7	0.053	0.043	0.039	0.119
13	Penta 99	3.8	2.2	NS	5.2	2.2	1.0	N S	4.9	2.5	1.3	N S	3.3	0.091	0.063	NS	0.120
14	Raj Bundi	3.9	2.2	NS	5.6	2.1	1.5	NS	7.5	2.7	2.2	NS	3.3	0.091	0.080	NS	0.125
15	Penta 99-1	2.3	NS	NS	3.9	1.8	NS	0.0	2.9	1.7	NS	NS	3.0	0.098	NS	NS	0.098
16	ES 99	2.9	NS	NS	5.4	1.9	NS	NS	7.6	1.5	NS	NS	3.3	0.070	NS	NS	0.139
17	ISH 32/8/1	2.5	1.6	NS	3.8	1.0	0.9	NS	5.1	1.0	1.0	NS	3.0	0.058	0.053	NS	0.101
18	Wardan S2	4.6	1.0	NS	7.6	1.0	0.5	NS	2.2	3.3	1.0	0.0	5.0	0.127	0.023	0.000	0.145
19	ISH 26/50/7	3.0	2.1	1.0	7.9	1.0	NR	NR	3.4	2.5	1.3	NL	5.7	0.103	0.071	0.019	0.155
20	ISH 32/34/1	4.1	2.0	1.5	4.8	3.5	1.1	0.4	4.5	3.5	1.6	NL	3.7	0.098	0.061	0.046	0.091
21	Multi-98-45	2.7	1.6	NS	6.4	1.3	0.7	NS	3.0	2.6	1.0	0.0	4.0	0.090	0.074	NS	0.138
22	ISH 34/5/1	2.8	1.5	NS	6.0	0.9	0.9	NS	3.2	2.8	2.0	NS	4.0	0.105	0.084	NS	0.108
23	Raj 49/50	NS	NG	NG	4.9	NS	NG	NG	2.8	NS	NG	NG	3.0	NS	NG	NG	0.096
24	T 44-4	3.1	0.5	0.6	8.1	1.5	NR	NR	14.1	2.5	NL	NL	5.0	0.079	0.017	0.019	0.240
25	T 45-1	2.4	2.4	2.1	10.0	1.4	1.8	0.8	14.0	2.0	3.0	3.0	5.3	0.065	0.101	0.093	0.181
26	T 5-90I-1	3.4	1.8	1.2	4.1	1.8	0.4	0.6	3.7	3.0	2.0	1.0	3.7	0.184	0.077	0.054	0.117
27	ISH 8020B	4.3	2.9	2.2	9.9	7.4	3.1	1.2	15.1	3.2	2.2	2.0	5.3	0.136	0.087	0.071	0.300
28	ISH 8020Y	3.0	1.1	NS	7.7	3.3	NS	NS	9.2	2.3	NL	NS	3.6	0.088	0.042	NS	0.180
29	ISH 5050B	3.8	1.7	2.4	5.9	2.2	0.9	1.1	8.3	3.2	2.0	1.5	4.3	0.103	0.063	0.090	0.121
30	ISH 5050Y	3.9	NS	NS	4.0	2.4	NS	NS	5.4	2.7	NS	NS	3.3	0.086	NS	NS	0.111
31	ISH 34/8B	5.5	2.7	1.6	10.7	3.4	1.4	0.5	10.0	4.2	2.2	1.0	5.7	0.220	0.094	0.064	0.215
32	ISH 34/8Y	4.8	2.0	2.0	8.9	5.2	1.2	0.8	9.9	4.5	1.5	2.0	6.3	0.207	0.102	0.071	0.282
33	T 5-90-I	5.6	4.1	1.8	7.6	4.2	1.8	1.1	10.1	3.7	4.2	1.5	5.7	0.229	0.345	0.077	0.217
34	T-9-90FM	4.0	3.3	1.4	7.7	5.6	2.4	NR	10.4	3.6	2.2	1.0	5.7	0.260	0.243	0.057	0.304

NR=No roots, NS= No survival, NL= No leaves

Fig. 7

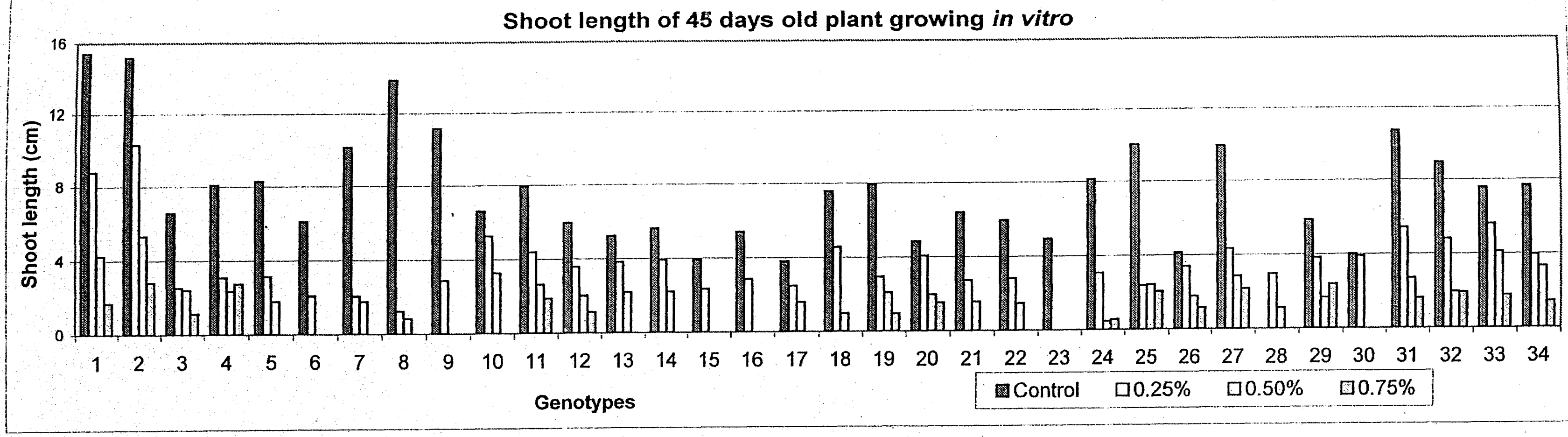
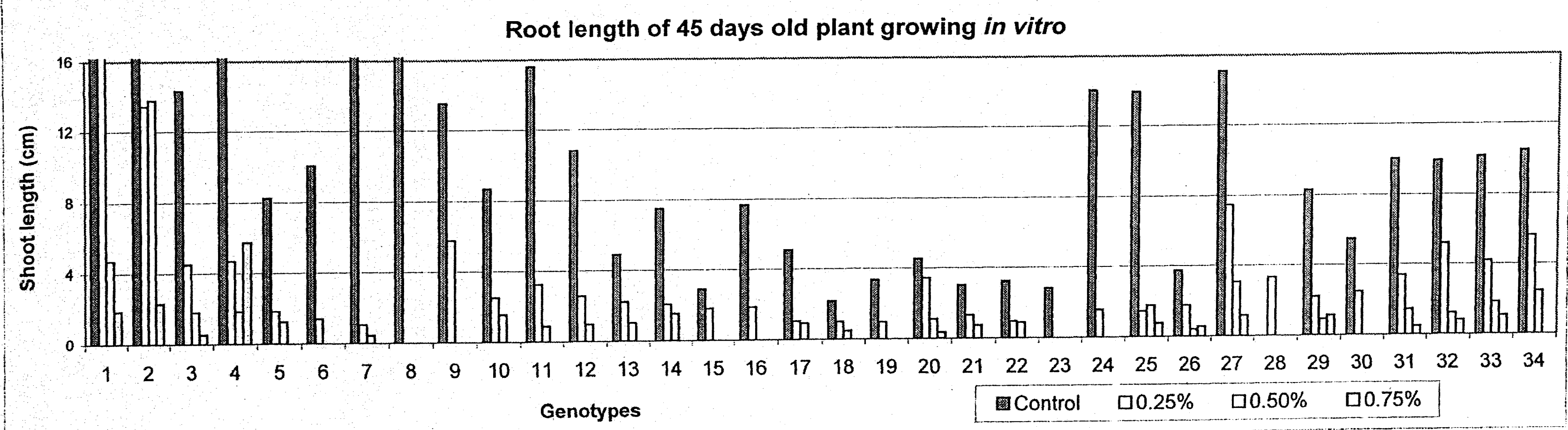


Fig. 8.



1. EC 329299, **2.** EC 318954, **3.** Wardan, **4.** EC 407709, **5.** EC 400976, **6.** EC 508311, **7.** EC 4017103, **8.** EC 400977, **9.** EC 401711, **10.** ISH 34/49, **11.** ISH 34/41, **12.** ISH 34/11, **13.** Penta 99, **14.** Raj Bundi, **15.** Penta 99-1, **16.** ES 99, **17.** ISH 32/8/1, **18.** Wardan S2, **19.** ISH 26/50/7, **20.** ISH 32/34/1, **21.** Multi-98-45, **22.** ISH 34/5/1, **23.** Raj 49/50, **24.** T 44-4, **25.** T 45-1, **26.** T 5-90I-1, **27.** ISH 8020B, **28.** ISH 8020Y, **29.** ISH 5050B, **30.** ISH 5050Y, **31.** ISH 34/8B, **32.** ISH 34/8Y, **33.** T 5-90-I, **34.** T-9-90FM.

Fig. 9.

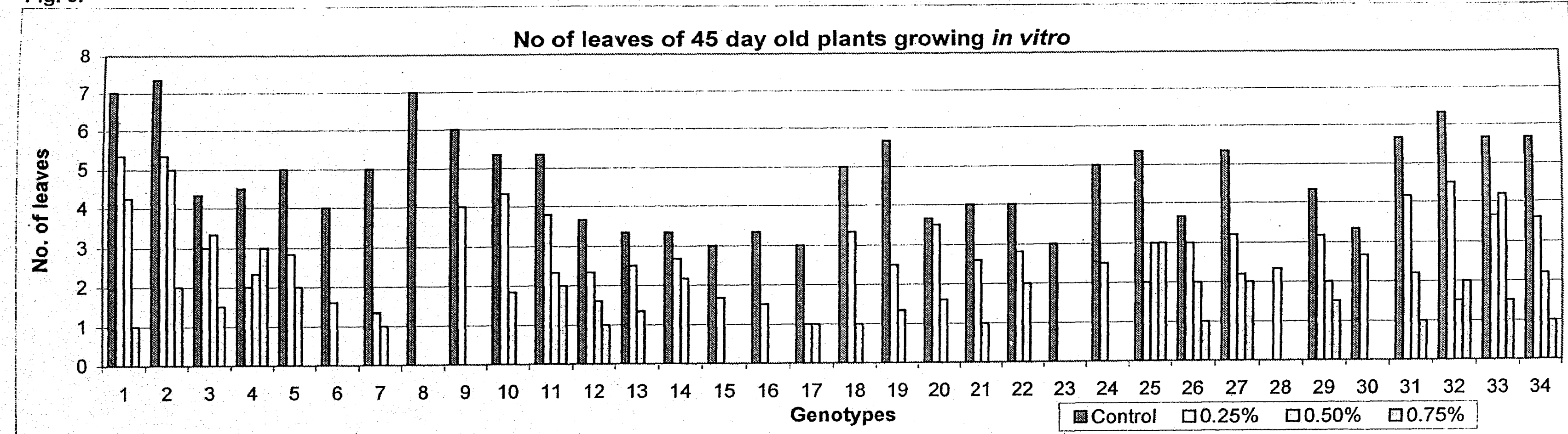
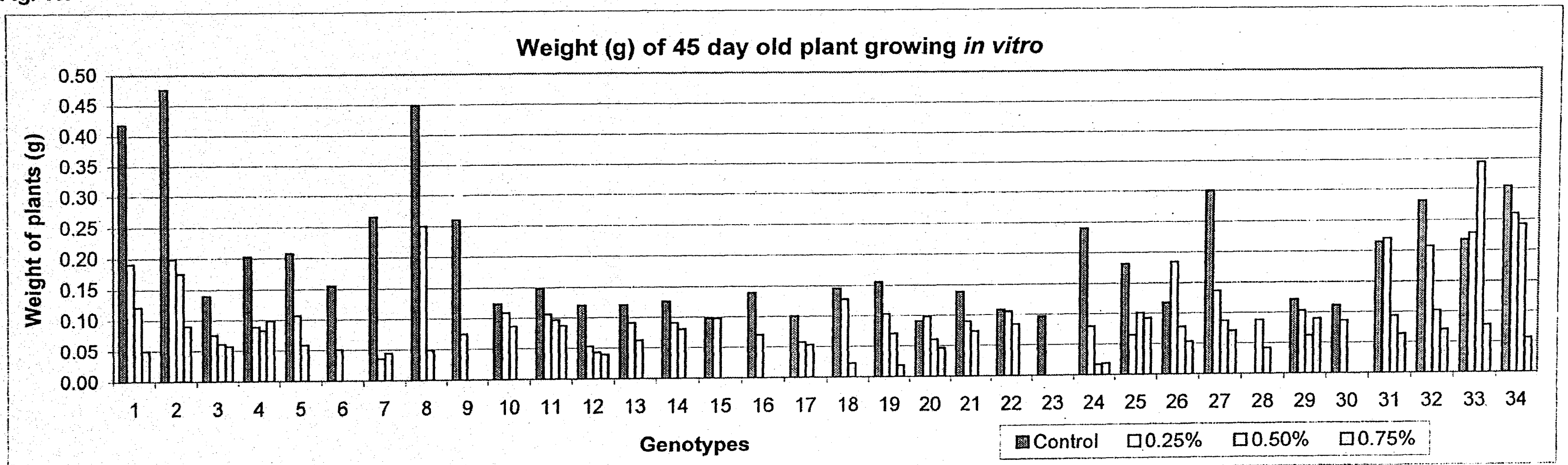


Fig. 10.



1. EC 329299, **2.** EC 318954, **3.** Wardan, **4.** EC 407709, **5.** EC 400976, **6.** EC 508311, **7.** EC 4017103, **8.** EC 400977, **9.** EC 401711, **10.** ISH 34/49, **11.** ISH 34/41, **12.** ISH 34/11, **13.** Penta 99, **14.** Raj Bundi, **15.** Penta 99-1, **16.** ES 99, **17.** ISH 32/8/1, **18.** Wardan S2, **19.** ISH 26/50/7, **20.** ISH 32/34/1, **21.** Multi-98-45, **22.** ISH 34/5/1, **23.** Raj 49/50, **24.** T 44-4, **25.** T 45-1, 26. T 5-90I-1, **27.** ISH 8020B, **28.** ISH 8020Y, **29.** ISH 5050B, **30.** ISH 5050Y, **31.** ISH 34/8B, **32.** ISH 34/8Y, **33.** T 5-90-I, **34.** T-9-90FM.

Root length: The average root length of the plants growing under control condition was 10.4 cm that reduced to 7.7 and 5.6 at 0.25% and 0.50% salinity respectively whereas at 0.75% salinity the plants were without roots.

Number of leaves: The average number of leaves in the plants under control condition was 5.7, which reduced to 3.6, 2.2 and 1.0 at 0.25%, 0.50% and 0.75% salinity respectively.

Weight of plants: The average biomass of the plants growing under control condition was 304 mg that reduced to 260, 243 and 57 mg at 0.25%, 0.50% and 0.75% salinity respectively.

C. Isozyme studies in seedlings growing *in vitro* under saline *vis-à-vis* normal condition

Isozyme studies were conducted using vertical PAGE gel electrophoresis to study the variations for the isozymic pattern of 3 enzymes i.e. SOD (Super Oxide Dismutase), PRX (Peroxidase) and Est. (Esterase) under 3 different levels of salinity (0.25%, 0.50% and 0.75%) compared with seedlings growing under control condition. The unambiguous bands were scored and given numbers starting from the place of origin. Bands were scored across the plates and genotypes. Banding patter for different enzymes is presented in Table 12-15.

C.1. Banding Pattern of different enzymes

Super oxide dismutase (SOD): The study showed presence of 4 bands identified as band No. 1 (RM 0.26), band No. 2 (RM 0.43), band No. 3 (0.60) and band No. 4 (RM 0.65) in saline as well as control conditions.

Peroxidase (PRX): The study revealed the presence of 11 bands, which were, identified as band No. 1 (RM 0.12), 2 (RM 0.16), 3 (RM 0.20), 4 (RM 0.32), 5 (RM 0.46), 6 (RM 0.48), 7 (RM 0.53), 8 (RM 0.56), 9 (RM 0.58), 10 (RM 0.60) and 11 (RM 0.64) in saline as well as control conditions.

Esterase: Esterase isozymic banding pattern revealed the presence of 18 bands, which were identified as band No. 1 (RM 0.24), 2 (RM 0.31), 3 (RM 0.37), 4 (RM 0.45), 5 (RM 0.48), 6 (RM 0.50), 7 (RM 0.54), 8 (0.56), 9 (RM 0.59), 10 (RM 0.64), 11 (RM 0.66), 12 (RM 0.69), 13 (RM 0.71), 14 (RM 0.73), 15 (0.77), 16 (RM 0.80), 17 (RM 0.82) and 18 (RM 0.84) in saline as well as control condition.

C.2. Isozyme banding pattern among genotypes under different salinity levels

EC 4017103

SOD - Total 4 bands were resolved of which 3 bands i.e. band No. 1, 2 and 3 were present in all the saline treatments as well as under control condition but the band No. 4 was present only in the susceptible plants at 0.25% salinity.

Peroxidase – Four peroxidase bands were present in this genotype of which band No. 1 and 9 were present in saline treatments as well as in control seedlings whereas band No. 3 and 8 were present only in the susceptible plants at 0.25% and 0.50% salinity.

Esterase – Esterase isozymic study revealed the presence of 9 bands of which band No. 4, 8, 10, 13, 14, 15 and 16 were present in all saline treatments as well as in control plants whereas band No. 6 and 9 were present only in the susceptible plants at 0.25% and 0.50% salinity levels.

EC 400977

SOD – In this genotype 4 bands were present of which band No. 1, 2 and 3 were present in both plants under stress as well as in control whereas band No. 4 was present in the susceptible plants only growing at 0.50% and 0.75% salinity levels.

Peroxidase –Peroxidase isozymic study revealed the presence of 3 bands of which band No. 1 and 9 were present in all saline treatments as well as in control plants whereas band No 3 was present in the susceptible plants growing at 0.50% and 0.75% salinity level.

Esterase – There were 8 bands present of which band No. 4, 10, 14, 15 and 16 were present in all saline treatments as well as in control plants whereas band No. 6, 9 and 13 were present only in susceptible plants growing at 0.25% and 0.50% salinity level respectively.

EC 401711

SOD- The study revealed the presence of 4 bands, which were present in both susceptible and resistant plants growing under different saline treatments as well as under control conditions.

Peroxidase – In this genotype 4 bands were resolved of which band No. 1 and 9 were present in both susceptible and resistant plants growing at 0.25% and 0.50% salinity as well as in seedlings growing under control condition. Band No. 8 was present in susceptible and resistant seedlings growing at 0.25% and 0.50% salinity but was absent under control condition. Band No. 3 was present only in the susceptible seedlings growing at 0.25% and 0.50% salinity.

Esterase – The study revealed the presence of 9 band of which band No. 4, 9, 10, 13, 14, 15 and 16 were present in susceptible and resistant seedlings growing at 0.25% and 0.50% salinity as well as in the control condition. Band No. 6 was present only in the susceptible plants growing at 0.50% salinity. Band No. 8 was present in both susceptible and resistant plants at 0.25% and 0.50% salinity but was absent under control condition.

ISH 34/41

Peroxidase – In this genotype band No. 1 and 9 were commonly present in both susceptible and resistant seedlings at all saline treatments as well as under control condition.

Esterase – The study revealed presence of 12 bands of which band No. 4, 5, 8, 9, 10, 13, 14, 15, 16 and 17 were common to stress as well as non- stress seedlings. Band No. 6 was present in the susceptible seedlings growing at 0.25%, 0.50% and 0.75% salinity level and absent under control condition. Band No. 7 was present only in the susceptible plants growing at 0.25% salinity level and the plants growing under control conditions. Band No. 14 was present only in the susceptible plants growing at 0.75% salinity.

ISH 34/11

Peroxidase – In this genotype 3 bands were present of which band No. 1 and 9 were common to plants growing under stress and control condition. Band no. 3 was present only in the susceptible seedlings at 0.25% salinity level.

Esterase – The isozymic study revealed the presence of 7 esterase bands of which band No. 4, 10, 13, 14, 15 and 16 were common to both susceptible and resistant plants growing under different salinity level as well as under control condition. Band No. 5 was present in the control seedlings only.

ES 99

SOD – In this genotype 4 bands were resolved and all the bands i.e. band No. 1, 2, 3 and 4 were common to both plants under stress and non-stress condition.

Peroxidase – The study revealed the presence of 3 bands of which band No. 1 and 9 were common to both stressed plants and control plants whereas band No. 8 was present only in the susceptible plants growing at 0.25% salinity level.

Esterase – The study revealed presence of 7 bands common to both the plants under stress and control.

ISH 5050Y

SOD – In this genotype 4 distinct bands were resolved and all the bands were present in both susceptible and resistant type of seedlings growing at 0.25% and 0.50% salinity as well as control condition.

Peroxidase – The study revealed the presence of 2 bands i.e. band No. 1 and 9 common to stresses plants as well as non-stressed plants.

Esterase – The study revealed the presence of 6 bands i.e. band No. 4, 6, 8, 10, 13 and 14. All the bands were present in both the susceptible and resistant type of seedlings growing at 0.25% and 0.50% salinity as well as under control condition.

Wardan S2

SOD – The study revealed the presence of 4 bands of which band no. 1, 2 and 3 were common to stressed and non- stressed seedlings whereas band no. 4 was present only in the control plants.

Peroxidase – In this genotype 5 distinct bands were present of which band No. 1, 2 and 9 were present in both stressed and non-stressed plants whereas band No. 5 and 8 were present in the susceptible plants growing at 0.25%, 0.50% and 0.75% salinity level.

Esterase – The study revealed the presence of 6 distinct bands of which band No. 4 and 13 were common to stressed and non- stressed plants. Band No. 10 and 14 were present only in the susceptible plants growing at 0.75% salinity, band No. 16 and 17 appeared in the susceptible seedlings growing at 0.25%, 0.50% and 0.75% salinity level.

ISH 26/50/7

SOD – The study revealed the presence of 3 distinct bands i.e. band no. 1, 2 and 3. All the 3 bands were common to susceptible and resistant plant types growing at 0.25% salinity and in the susceptible seedlings at 0.50% and 0.75% salinity, as well as under control condition.

Peroxidase – In this genotype 5 distinct bands were resolved of which band no. 1, 2 and 9 were common to stress as well as non- stressed plants whereas band no. 5 was present in both susceptible and resistant seedlings growing at 0.25% salinity, in the susceptible seedlings growing at 0.50%, 0.75% and in the seedlings growing under control.

Esterase – The study revealed the presence of 4 bands of which band no. 4, 13 and 16 were present in both treated and non-treated plants whereas band no. 14 was present in both the susceptible and resistant plants growing at 0.25% salinity, in the susceptible plants growing at 0.50%, 0.75% salinity and in plants growing under control condition.

EC 329299

SOD – The study revealed the presence of 4 distinct bands across the plates. The band no. 1, 2, 3 and 4 were present in all the saline treatments in both susceptible and resistant plant as well as in plants growing under control condition.

Peroxidase – In this genotype 6 distinct bands were present across the plate of which band no. 1 and 9 were common to all the saline treatments in both susceptible and resistant plants and in the seedlings growing under control condition. Band No. 3 was absent in the resistant plants at 0.25% and under control condition whereas it was present in both susceptible and resistant plants growing at 0.50% and 0.75% salinity. Band No. 8 was present only in the resistant plants at 0.25% and in susceptible plants at 0.50% salinity. Band No. 10 and 11 were present only in the susceptible plants growing at 0.50% and 0.75% salinity and in the resistant plants growing at 0.50% salinity.

Esterase – The study revealed the presence of 7 distinct bands across the plates of which band no. 4, 10, 13, 14 and 16 were common in both resistant and susceptible plants in all the salinity treatments as well as under control condition. Band No. 6 was present only in the resistant plants growing at 0.50% and 0.75% salinity treatments. Band No. 17 was present in the resistant seedlings growing at 0.25%, 0.50% and 0.75% salinity level as well as in the susceptible plants growing at 0.75% salinity.

EC 318954

SOD – The study revealed the presence of 4 distinct bands i.e. band no. 1, 2, 3 and 4 commonly present in susceptible and resistant plants in all the saline treatments as well as under control condition.

Peroxidase – In this genotype 6 bands were resolved of which band no. 1 and 9 were common to stressed and non- stressed plants. Band No. 3 was present only in the susceptible plant growing at 0.50% and 0.75% salinity levels. Band No. 8 was present only in the resistant plants at 0.75% salinity and under control conditions. Band No. 10 and 11 appeared in both susceptible and resistant plants growing at 0.50% and 0.75% salinity level and were absent in 0.25% salinity and under control.

Esterase – The study revealed the presence of 8 distinct bands across the plate of which band no. 4, 10, 13, 14 and 15 were common to both susceptible and resistant plants growing at all salinity treatments and under control condition. Band No. 6 and 8 appeared only in the resistant plants growing at 0.75% salinity level whereas band no. 17 was present only in the susceptible plants growing at 0.75% salinity.

Wardan

SOD – In this genotype 4 SOD bands were resolved i.e. band no. 1, 2, 3 and 4. All the 4 bands were common to both susceptible and resistant seedlings growing in all the saline treatments and under control condition.

Peroxidase – The study revealed the presence of 6 distinct bands across the plates of which band no. 1 was present in stressed as well as non- stressed plants. Band No. 2 was present only in the susceptible plants growing at 0.25% salinity. Band No. 3 was present in the susceptible plants growing at 0.25%, 0.50% and 0.75% salinity and in the resistant plants growing at 0.50% salinity. Band No. 8 was present only in the resistant plants growing at 0.25% salinity. Band No. 9 was absent only in the control plants. Band No. 10 was present only in the resistant plants growing at 0.50% salinity.

Esterase – The study revealed the presence of 10 bands across the plates of which band no. 4, 6, 10, 14 and 16 were common in both susceptible and resistant plants growing in all the salinity treatments and under control condition. Band No. 7 was present only in the susceptible plants growing under 0.50% salinity. Band No. 8 was present only in the resistant plants growing at 0.50% salinity and in the susceptible plants at 0.75% salinity. Band no. 13 and 17 were absent only in the plants growing under control. Band No. 15 was absent only in the susceptible seedlings growing at 0.25% salinity and under control condition.

EC 407709

SOD – In this genotype 4 distinct SOD bands were resolved i.e. band no. 1, 2, 3 and 4. All the 4 bands were common to susceptible and resistant type of seedlings under stress and control.

Peroxidase – The study revealed the presence of 5 distinct bands of which band no. 1, 2 and 9 were common in both the susceptible and resistant type of seedlings growing at all saline treatment and under control condition. Band No. 3 was present in the susceptible seedlings at 0.25%, and 0.50% salinity level and in the resistant seedlings at 0.50% and 0.75% salinity. Band no. 8 was present in the susceptible seedlings growing at 0.25%, 0.50% and 0.75% salinity and in the resistant plants growing at 0.50% salinity.

Esterase – The isozymic study in this genotype revealed the presence of 12 distinct bands of which band no. 4, and 10 were common in all stressed as well as non- stressed plants. Band No. 1 was present only in the susceptible plants growing under 0.75% salinity. Band no. 5 was present only in the resistant plants growing at 0.25% and 0.50% salinity level. Band No. 6 was present only in the susceptible plants growing at 0.50% and 0.75%

salinity level. Band no. 8 was present only in the resistant plants at 0.75% salinity and in the control plants. Band no. 9 was present only in the susceptible plants growing at 0.75% salinity. Band no. 12 was present only in the resistant plants growing at 0.50% salinity. Band no. 13 was absent only in the resistant plants growing at 0.25% salinity and in plants growing under control. Band no. 14 was present only in the susceptible plants growing at 0.25% and 0.50% salinity and in the resistant plants at 0.50% salinity. Band no. 15 and 16 were absent only in the plants growing under control condition.

EC 400976

SOD – In this genotype the isozymic study revealed the presence of 4 distinct SOD bands i.e., band no. 1, 2, 3 and 4. All the bands were present in the stressed as well as non-stressed plants.

Peroxidase – The study revealed the presence of 5 bands of which band no. 1 and 9 were common to stressed as well as non- stressed plants whereas band no. 2 was present only in the susceptible type of plants at 0.25% salinity. Band no. 3 was present only in the susceptible plant growing at 0.50% and 0.75% salinity. Band no. 8 was present only in the susceptible plant growing at 0.25%, 0.50% and 0.75% salinity level.

Esterase – The isozymic study revealed the presence of 12 distinct bands of which bands no. 4, 10, 12, 13, 14, 15 and 16 were common in all saline stressed and non – stressed plants. Bands no. 6 was present only in the resistant seedlings growing at 0.25% salinity and in the susceptible seedlings growing at 0.50% and 0.75% salinity. Band no. 8 and 18 were present only in the susceptible seedlings growing at 0.50% and 0.75% salinity. Band no. 9 was absent in the control plants but present in all the saline treatments. Bands no. 11 was present in the susceptible plants growing at 0.25%, 0.50% and 0.75% salinity level

EC 508311

SOD – In this genotype isozymic study revealed the presence of 4 distinct bands. All the bands i.e., band no. 1, 2, 3 and 4 were common to stressed and non- stressed plants.

Peroxidase – The study revealed the presence of 7 distinct bands across the plate of which band no. 1, 2, 3 and 9 were common to stressed and non- stressed plants, whereas band no. 8, 10 and 11 were present in the susceptible plants growing at 0.25% and 0.50% salinity levels.

Esterase – The isozymic study revealed the presence of 12 esterase bands across the plate of which bands no. 4, 6, 7, 9, 10, 12, 15 and 16 were present in all stressed and non-stressed plants. Band no. 2 was present only in the control plants. Band no. 8, 14 and 15 were present in the susceptible plants growing at 0.25% and control conditions.

ISH 32/34/1

SOD – In this genotype the isozymic banding pattern revealed the presence of 4 bands of which band no. 1, 2, and 3 were common to stressed and non- stressed plants. Band no. 4 was present only in the resistant plants growing at 0.25% salinity.

Peroxidase – The study revealed the presence of 6 distinct bands across the plate of which band no. 1, 2, and 9 were common to stressed and non- stressed plants, whereas band no. 8, was present in the susceptible and resistant plants growing at 0.50% salinity level. Band no. 10 and 11 were present only in the susceptible plants growing at 0.50% and 0.75% salinity level.

Esterase – The isozymic study revealed the presence of 5 esterase bands across the plate of which bands no. 4, 10, 13, 14, and 16 were present in both the susceptible and resistant plants growing in all treatments and control condition.

Multi-98-45.

SOD – In this genotype the isozymic study revealed the presence of 3 distinct bands across the plates. The band no. 1, 2 and 3 were present in all the stressed plants as well as non- stressed plants.

Peroxidase - The study revealed the presence of 10 distinct bands of which band No. 1, 2 and 9 were common in both susceptible and resistant plants in all treatments and control plants. Band No. 3 and 7 were present only in the susceptible plants growing at 0.25% and 0.50% salinity levels. Band No. 4 and 5 were present in the susceptible and resistant plants growing at 0.25% salinity level. Band no. 8 was present only in the susceptible plants growing at 0.25% salinity. Band No. 10 and 11 were present in the susceptible plants growing at 0.25% and 0.50% salinity and in the resistant plants at 0.25% salinity.

Esterase – The isozymic study revealed the presence of 7 distinct bands across the plates of which band no. 4, 6, 13 and 15 were common to stressed and non- stressed plants whereas band no. 10 and 16 were present in the susceptible and resistant plants at 0.25% salinity and under control condition. Band no. 14 was present only in the susceptible and resistant plants growing at 0.25% salinity level.

ISH 34/5/1

SOD – In this genotype the isozymic study revealed the presence of 3 distinct bands of which all the 3 bands i.e., band no. 1, 2 and 3 were present in stressed and non- stressed plants.

Peroxidase - The study revealed the presence of 9 distinct bands of which band No. 2 and 9 were common to both the susceptible and resistant plants growing under saline

treatment and control condition. Band no.1 and 4 was present only in the susceptible and resistant plants growing at 0.25% salinity and band no. 4 in the susceptible plants growing at 0.75% salinity. Band No. 5 was present in the susceptible plants growing at 0.25% and 0.50% salinity. Band No. 6 was present in the susceptible plants growing at 0.75% salinity. Band No.7 was present only in the susceptible plants growing at 0.25% salinity. Band No. 10 and 11 were present in the susceptible plants at 0.50% and 0.75% salinity and under control condition.

Esterase – The study reveled the presence of 7 distinct bands across the plate of which band No. 4, 6, 10, 13, 15 and 16 were common in both susceptible and resistant plants growing under salinity treatments and under control condition, whereas band No. 8 was present only in the susceptible plants at 0.25% and 0.75% salinity levels.

T 5-90I-1

SOD – In this genotype the isozymic study revealed the presence of 4 distinct bands across the plates. All the 4 bands i.e., 1, 2, 3 and 4 were present in stressed and non stressed plants.

Peroxidase – The study revealed the presence of 2 bands i.e., band No. 1 and 9 only and both were common in the treated and non- treated plants.

Esterase – The study revealed the presence of 5 distinct bands of which band No. 4, 5, 13 and 14 were common in stressed and non- stressed plants whereas band No. 16 was present in the susceptible plants at 0.25% and 0.50%, in the resistant plants at 0.25% salinity and in the control plants.

T 45-1

SOD - In this genotype 4 bands were resolved and all the bands i.e., 1, 2, 3 and 4 were common to stressed and non- stressed plants

Peroxidase – The study revealed the presence of 2 bands i.e., band No. 1 and 9, which were present in both stressed and under control plants.

Esterase - The isozymic study revealed the presence of 6 distinct bands of which band No. 4, 13, 14 and 16 were common to stressed and non- stressed plants whereas band No. 6 was present in the susceptible and resistant plants at 0.25% and 0.75% salinity and under control. Band No. 7 was present only under control condition.

T 44-4

SOD – In this genotype the isozymic study revealed the presence of 4 distinct bands i.e., band No. 1, 2, 3 and 4. These bands were common to plants growing in saline treatments and control.

Peroxidase – The study revealed the presence of only 2 bands i.e., band No. 1 and 9. Both these bands were common to stressed as well as non- stressed plants.

Esterase – The isozymic study revealed the presence of 4 bands of which band No. 4, 13 and 14 were common to both stressed and non- stressed plants whereas band No. 16 was absent only under control.

ISH 5050B

SOD – In this genotype 4 bands were resolved i.e., band No. 1, 2, 3 and 4. All the bands were common to stressed and non- stressed plants.

Peroxidase – The study revealed the presence of 3 bands of which band No. 1 and 9 were common to both susceptible and resistant plants in all saline treatments and control condition. Band No. 8 was present only in the resistant plants at 0.25% salinity.

Esterase – The isozymic banding pattern revealed the presence of 6 distinct bands of which band No. 4, 10, and 13 were common to both susceptible and resistant plants at all salinity levels and under control condition. Band No.9 was present only in the resistant plants at 0.25% salinity, in the susceptible plants at 0.50% salinity and in the control plants. Band no. 14 and 16 were present in the susceptible plants growing at 0.25% and 0.50% salinity and in the resistant seedlings at 0.25% salinity level.

ISH 8020Y

SOD – In this genotype the isozymic banding pattern revealed the presence of 4 bands i.e., band No. 1, 2, 3 and 4 and all the bands were invariably present in plants growing under control or saline conditions.

Peroxidase – The study revealed the presence of 4 bands of which band No. 1 and 9 were common to stressed and non- stressed plants whereas band No. 8 was present only in the resistant and susceptible plants growing at 0.25% and 0.50% salinity and under control condition. Band No. 11 was present only in the susceptible plants growing at 0.25% salinity.

Esterase – The isozymic banding pattern revealed the presence of 8 distinct bands across the plates of which band No. 4, 9, 10, 13 and 14 were common to both susceptible and resistant plants at different salinity levels and under control condition. Band No. 2 and 6 was present only in the plants growing under control condition. Band No. 16 was present in the susceptible plants growing at 0.25% and 0.50% salinity levels.

ISH 34/8B

SOD – In this genotype 4 distinct bands were resolved of which 3 bands i.e., No. 1, 2 and 3 were common to both susceptible and resistant plants growing at different salinity levels

Table 12. SOD, Esterase and PRX banding pattern in 20 day old seedlings of Egyptian clover genotypes growing in vitro

	SOD				PRX										
Band No.	**1**	**2**	**3**	**4**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**
RM value	0.26	0.43	0.6	0.65	0.12	0.16	0.2	0.32	0.46	0.48	0.53	0.56	0.58	0.6	0.64
E C 4017103															
0.25% S	pp	ppp	ppp	p	p		p					p	p		
0.50% S	pp	ppp	ppp		p		p					p	pp		
Control	pp	ppp	ppp		p								p		
E C 400977															
0.25% S	pp	ppp	ppp		p								p		
0.50% S	ppp	ppp	ppp	p	p		p						p		
0.75% S	pp	ppp	ppp	p	p		p						p		
Control	pp	ppp	ppp		p[								p		
E C 401711															
0.25% S	ppp	ppp	ppp	p	p		p					p	p		
0.25% R	pp	ppp	ppp	p	p							p	p		
0.50% S	pp	ppp	ppp	p	p		p					p	p		
Control	pp	ppp	ppp	p	p								p		
ISH 34/41															
0.25% S					p								p		
0.50% S					p								p		
0.75% S					p								p		
Control					p								p		
ISH 34/11															
0.25% S					pp		p						p		
0.25% R					p								p		
0.50% S					p								p		
0.50% R					p								p		
0.75% S					pp								p		
Control					p								p		
ES 99															
0.25% S	ppp	ppp	ppp	pp	p							p	p		
Control	ppp	ppp	ppp	pp	p								p		
ISH 32/8/1															
0.25% S	ppp	ppp	ppp	pp	p								p		
0.50% S	ppp	ppp	ppp	pp	p							p	p		
Control	ppp	ppp	ppp	pp	p								p		
ISH 5050Y															
0.25% S	ppp	ppp	ppp	pp	p								p		
0.25% R	ppp	ppp	ppp	pp	p								p		
0.50% S	ppp	ppp	ppp	pp	p								p		
Control	ppp	ppp	ppp	pp	p								p		

	Est																	
Band No.	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**
RM value	0.24		0.37	0.45	0.48	0.5	0.54	0.56	0.59	0.64	0.66	0.69	0.71	0.73	0.77	0.8	0.82	0.84
E C 4017103																		
0.25% S				ppp		p		p	p	p			p	p	p	p		
0.50% S				ppp		p		p	p	p			pp	p	p	p		
Control				pp				p		p			p	p	p	p		
E C 400977																		
0.25% S				pp					p	p			p	p	p	p		
0.50% S				pp		p				p			p	pp	p	p		
0.75% S				pp						pp				pp	p	p		
Control				pp						p				p	p	p		
E C 401711																		
0.25% S				pp				p	p	p			p	ppp	p	p		
0.25% R				pp				p	p	p			p	p	p	p		
0.50% S				pp		p		p	p	pp			p	p	p	p		
Control				pp					p	p			p	p	p	p		
ISH 34/41																		
0.25% S				ppp	pp	p	p	p	p	pp			pp		p	pp	p	
0.50% S				ppp	pp	p		p	p	pp			pp		p	pp	p	
0.75% S				pp	pp	p		p	p	pp			p	p	p	pp	p	
Control				ppp	pp		p	p	p	pp			p		p	p	p	
ISH 34/11																		
0.25% S				ppp						p			p	p	p	pp		
0.25% R				ppp						pp			p	pp	p	pp		
0.50% S				ppp						pp			p	p	p	p		
0.50% R				ppp						pp			p	p	p	pp		
0.75% S				ppp						pp			p	p	p	pp		
Control				ppp	p					pp			p	p	p	pp		
ES 99																		
0.25% S				pp		p				p			p	p	p	p		
Control				pp		p				p			p	p	p	p		
ISH 32/8/1																		
0.25% S				pp		p			p	p			p	pp	p	p		
0.50% S				pp		p			p	p			p	pp	p	p		
Control				ppp		p		p	p	p			p	pp	pp	pp		
ISH 5050Y																		
0.25% S				ppp		p		p		pp			ppp	pp				
0.25% R				ppp		p		p		pp			pp	pp				
0.50% S				ppp		p		p		p			pp	pp				
Control				ppp		p		p		p			p	p				

p=present light band, pp=medium intensity band, ppp=dark band, pppp= very dark band, blank=absent

Table 13. SOD, Esterase and PRX banding pattern in 20 day old seedlings of Egyptian clover genotypes growing in vitro

Genotypes	SOD Band No. 1	SOD 2	SOD 3	SOD 4	PRX 1	PRX 2	PRX 3	PRX 4	PRX 5	PRX 6	PRX 7	PRX 8	PRX 9	PRX 10	PRX 11	Est 1	Est 2	Est 3	Est 4	Est 5	Est 6	Est 7	Est 8	Est 9	Est 10	Est 11	Est 12	Est 13	Est 14	Est 15	Est 16	Est 17	Est 18
RM value	0.26	0.43	0.6	0.65	0.12	0.16	0.2	0.32	0.46	0.48	0.53	0.56	0.58	0.6	0.64	0.24		0.37	0.45	0.48	0.5	0.54	0.56	0.59	0.64	0.66	0.69	0.71	0.73	0.77	0.8	0.82	0.84
EC 329299																																	
0.25% R	ppp	ppp	ppp	pp	p							p	p						pp						p			p	p		p	p	
0.50% S	ppp	ppp	ppp	pp	p		p					p	p	p	p				pp						p			p	p		pp		
0.50% R	ppp	ppp	ppp	pp	p		p						p	p	p				pp		p				p			p	p		pp	p	
0.75% S	ppp	ppp	ppp	pp	p		p						pp	p	p				pp						p			p	p		pp	p	
0.75% R	ppp	ppp	ppp	pp	p		p						p						pp		p				p			p	p		pp	p	
Control	ppp	ppp	ppp	pp	p								p						pp						p			p	p		p		
EC 318954																																	
0.25% R	ppp	ppp	ppp	p	p								p						pp						p			p	p		p		
0.50% S	ppp	ppp	ppp	p	p		p						pp	p	p				pp						p			p	p		pp		
0.50% R	ppp	ppp	ppp	p	p								p	p	p				pp						p			p	p		p		
0.75% S	ppp	ppp	ppp	p	p		p						pp	p	p				pp						p			p	p		pp	p	
0.75% R	ppp	ppp	ppp	p	p							p	p	p	p				pp		p		p		p			p	p		p		
Control	ppp	ppp	ppp	pp	p							p	p						pp						p			p	p		p		
Wardan																																	
0.25% S	pp	ppp	ppp	pp	p	p	pp						pp						ppp		p				p			p	p		pp	p	
0.25% R	pp	ppp	ppp	pp	p							p	p						ppp		p				p			p	p	p	pp	p	
0.50% S	pp	ppp	ppp	pp	p		pp						p						ppp		p	p			p			p	p	p	pp	p	
0.50% R	pp	ppp	ppp	pp	p		p						p	p					ppp		p		p		p			p	pp	p	pp	p	
0.75% S	pp	ppp	ppp	pp	p		p						pp						ppp		p		p		p			p	pp	p	pp	p	
Control	pp	ppp	ppp	pp	p														ppp		p				p				p		p		
E C 407709																																	
0.25% S	ppp	ppp	ppp	pp	pp	p	p					p	p						pp						p			p	p	p	p		
0.25% R	ppp	ppp	ppp	pp	pp	p							p						pp	p					p					p	p		
0.50% S	ppp	ppp	ppp	pp	pp	p	p					p	p						pp		p				p			p	p	p	p		
0.50% R	ppp	ppp	ppp	pp	pp	p	p					p	p						pp	p					pp		p	p	p	p	p		
0.75% S	ppp	ppp	ppp	pp	pp	p						p	p			pp			pp		p			p	p			p		p	p		
0.75% R	ppp	ppp	ppp	pp	pp	p	p						p						pp				p		p			p		p	p		
Control	ppp	ppp	ppp	pp	p	p							p						pp				p		p								
E C 400976																																	
0.25% S	ppp	ppp	ppp	pp	pp	pp						p	p						pp					p	p	p	p	p	pp	p	p		
0.25% R	ppp	ppp	ppp	pp	p								p						pp		p			p	p		p	p	p	p	p		
0.50% S	ppp	ppp	ppp	pp	p		p					p	p						pp		p		p	p	pp	p	p	pp	pp	p	pp		p
0.75% S	ppp	ppp	ppp	pp	p		p					p	p						pp		p		p	p	pp	p	p	pp	pp	p	pp		p
Control	ppp	ppp	ppp	pp	p								p						pp						p		p	p	p	p	p		
E C 508311																																	
0.25% S	ppp	ppp	ppp	p	pp	p	p					p	p	p	p				ppp		pp	p	p	p	pp			p	p	p	p	p	
0.50% S	ppp	ppp	ppp	p	pp	pp	p					p	p	p	p				ppp		p	p		p	pp			p			p	p	
Control	ppp	ppp	ppp	p	pp	p	p						p					pp	ppp		p	p	p	p	pp			p	p	p	p	p	

Table 14. SOD, Esterase and PRX banding pattern in 20 day old seedlings of Egyptian clover genotypes growing in vitro

	SOD					PRX												Est																	
Band No.	1	2	3	4		1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RM value	0.26	0.43	0.6	0.65		0.12	0.16	0.2	0.32	0.46	0.48	0.53	0.56	0.58	0.6	0.64		0.24		0.37	0.45	0.48	0.5	0.54	0.56	0.59	0.64	0.66	0.69	0.71	0.73	0.77	0.8	0.82	0.84
0.25% S	pp	ppp	ppp			pp	pp			p			p	pp							p									p			p	p	
0.50% S	pp	ppp	ppp			pp	pp			pp			p	pp							pp									p			p	p	
0.75% S	pp	ppp	ppp			pp	pp			pp			pp	pp							p						p			p	p		p	p	
Control	pp	ppp	ppp	p		p	p							p							p									p					
ISH 26/50/7																																			
0.25% S	pp	ppp	ppp			p	pp			p				p							p									p	p		p		
0.25% R	pp	ppp	ppp			p	p			p			p	p							p									p	p		p		
0.50% S	pp	ppp	ppp			p	p			p				p							p									p	p		p		
0.75% S	pp	ppp	ppp			pp	pp			p				p							pp									p	p		pp		
Control	pp	ppp	ppp			p	p							p							p									p			p		
ISH 32/34/1																																			
0.25% S	pp	ppp	ppp			pp	pp							p							p						p			p	p		p		
0.25% R	pp	ppp	ppp	p		p	pp							p							p						p			p	p		p		
0.50% S	pp	ppp	ppp			p	p						p	p	p	p					p						p			p	p		p		
0.50% R	pp	ppp	ppp			p	p						p	p							p						p			p	p		p		
0.75% S	pp	ppp	ppp			pp	pp							p	p	p					p						p			p	p		p		
Control	pp	ppp	ppp			p	p							p							p						p			p	p		p		
Multi-98-45																																			
0.25% S	pp	pp	ppp			ppp	pp	p	p	p		p	p	pp	p	p					pp		p				p			p	p	p	p		
0.25% R	pp	ppp	ppp			ppp	pp		p	p				p	p	p					pp		p				p			p	p	p	p		
0.50% S	pp	ppp	ppp			ppp	pp	p				p		p	p	p					pp		p							p		p			
Control	pp	ppp	ppp			p	p							p							pp		p				p			p		p	p		
ISH 34/5/1																																			
0.25% S	pp	ppp	ppp			pp	pp		p	p		pp		p							pp		p		p		p			p		p	p		
0.25% R	pp	ppp	ppp			p	pp		p					p							pp		p				p			p		p	p		
0.50% S	pp	ppp	ppp				pp			p				p	p	p					pp		p				p			p		p	p		
0.75% S	pp	ppp	ppp				p		p		p			pp	p	p					pp		p		p		p			pp		p	pp		
Control	pp	ppp	ppp				p							p	p	p					pp		p				p			p		p	p		
T 5-90I-1																																			
0.25% S	ppp	ppp	ppp	p		p								p							pp		p							p	p		p		
0.25% R	ppp	ppp	ppp	p		p								p							p		p							p	p		p		
0.50% S	ppp	ppp	ppp	p		p								p							p		p							p	p		p		
0.50% R	ppp	ppp	ppp	p		p								p							p		p							p	p				
Control	ppp	ppp	ppp	p		p								p							pp		p							p	p		p		
T 45-1																																			
0.25% S	ppp	ppp	ppp	p		p								p							p		p							p	p		p		
0.25% R	ppp	ppp	ppp	p		p								p							p									p	p		p		
0.50% S	ppp	ppp	ppp	p		p								p							p									p	p		p		
0.50% R	ppp	ppp	ppp	p		p								p							p		p							p	p		p		
Control	ppp	ppp	ppp	p		p								p							p		p	p						p	p		p		
T 44-4																																			
0.25% S	ppp	ppp	ppp	p		p								p							p									p	p		p		
0.25% R	ppp	ppp	ppp	p		p								p							p									p	p		p		
0.50% S	ppp	ppp	ppp	p		p								p							p									p	p		p		
Control	ppp	ppp	ppp	p		p								p							p									p	p				

Table15. SOD, Esterase and PRX banding pattern in 20 day old seedlings of Egyptian clover genotypes growing in vitro

	SOD					PRX												Est																	
Band No.	1	2	3	4		1	2	3	4	5	6	7	8	9	10	11		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
RM value	0.26	0.43	0.6	0.65		0.12	0.16	0.2	0.32	0.46	0.48	0.53	0.56	0.58	0.6	0.64		0.24		0.37	0.45	0.48	0.5	0.54	0.56	0.59	0.64	0.66	0.69	0.71	0.73	0.77	0.8	0.82	0.84
ISH 5050B																																			
0.25% S	pp	ppp	ppp	p		pp							p	p							p						p			p	p		p		
0.25% R	pp	ppp	ppp	p		pp								p							p					p	p			p	p		p		
0.50% S	pp	ppp	ppp	p		pp								p							p					p	p			p	p		p		
Control	pp	ppp	ppp	p		pp								p							p					p	p			p					
ISH 8020B																																			
0.25% S	pp	ppp	ppp	p		pp								p		p					pp						p			p	p				
0.25% R	pp	ppp	ppp	p		pp							p	p							pp					p	p			p	p				
0.50% S	pp	ppp	ppp	p		pp							p	p							pp						p			p	p				
Control	pp	ppp	ppp	p		pp							p	p						p	pp		p			p	p			p	p				
ISH 8020Y																																			
0.25% S	pp	ppp	ppp	p		pp								p							pp					p	p			p	p		p		
0.25% R	pp	ppp	ppp	p		pp								p							pp					p	p			p	p				
0.50% S	pp	ppp	ppp	p		pp							p	p							pp					p	p			p	p		p		
Control	pp	ppp	ppp	p		pp								p		p			pp		pp		p			p	p			p	p				
ISH 34/8B																																			
0.25% S	pp	ppp	ppp																																
0.25% R	pp	ppp	ppp	p																															
0.50% S	pp	ppp	ppp																																
0.50% R	pp	ppp	ppp	p																															
0.75% S	pp	ppp	ppp																																
Control	pp	ppp	ppp																																
ISH 34/8Y																																			
0.25% S	pp	ppp	ppp																																
0.25% R	pp	ppp	ppp																																
0.50% S	pp	ppp	ppp																																
0.50% R	pp	ppp	ppp																																
0.75% S	pp	ppp	ppp																																
Control	pp	ppp	ppp	p																															

and under control plants whereas band No. 4 was present in the resistant plants growing at 0.25% and 0.50% salinity levels.

ISH 34/8Y

SOD - In this genotype 4 band were resolved of which 3 bands i.e., band No. 1,2 and 3 were common to both susceptible and resistant plants growing at different salinity levels and under control condition whereas band No. 4 was present in the control plants only.

D. Biochemical studies in seedlings growing in pots under saline *vis-à-vis* normal condition

Native protein banding, SDS protein banding and K and Na ion estimation was carried out on eight selected genotypes growing in pots.

D.1. Native Protein

The electrophoretic banding pattern of the seedlings of the selected genotypes of *Trifolium alexandrinum* was carried out using Native PAGE. The study revealed the presence of 22 distinct protein bands. The bands were scored and given numbers based on relative mobility, starting from the place of origin. The band were identified as band No.1 (RM 0.06), 2 (RM 0.08), 3 (RM 0.10), 4 (RM 0.107), 5 (RM 0.15), 6 (RM 0.25), 7 (RM 0.31), 8 (RM 0.36), 9 (RM 0.46), 10 (RM 0.48), 11 (RM 0.51), 12 (RM 0.53), 13 (RM 0.55), 14 (RM 0.56), 15 (RM 0.52), 16 (RM 0.60), 17 (RM 0.62), 18 (RM 0.65), 19 (RM 0.69), 20 (RM 0.73), 21 (RM 0.74) and 22 (RM 0.79) (Table 16).

Wardan: The banding pattern revealed the presence of 20 distinct bands, band No.1 and 7 were absent on this genotype. The common bands were 4, 5, 8, 9, 10, 11, 13, 14, 15, 16, 17, 19, 20, 21 and 22. These bands were common across all the four saline treatments i.e. 0.25%, 0.50%, 0.75% and 1% and under control condition. Band No. 2 and 3 were present in all the saline treatments but absent in the plants growing under control condition.

Band No.6 and 18 were absent in the plants under salinity but present in the control plants. Band No.12 was absent at 0.25% salinity and control condition but present in the plants growing at 0.50%, 0.75% and 1% salinity.

EC 407709: The protein PAGE study revealed the presence 20 bands of which band No. 4,5,8,9,10,11, 13,14,15,16,17,19.20,21 and 22 were common at all salinity levels and control condition whereas Band No.2 was absent only under control condition. Band No.3 was present only at 1% salinity. Band No.6 was present at 0.25% and 1% salinity and

under control condition. Band No. 12 was present at 1% salinity and control plants whereas band No.18 was absent only at 0.50% salinity.

T 45-1: The Native PAGE study in this genotype revealed the presence of 19 distinct bands of which band No.3, 4,5,8,9,10,11,12, 14,16,18,19, 21 and 22 were common to treated and non- treated plants. The band No.1 and 2 were present only in the 0.75% and 1% salinity level. Band No.6 and 13 were present only in the plants growing at 0.25% salinity whereas band No. 17 was present at 0.25% salinity and control condition only.

ISH 8020B: The protein banding pattern study in this genotype revealed the presence of 17 distinct bands of which band No. 3,4,5,8,9,10,12,14,16,18,19,21 and 22 were common to both treated and non-treated plants, whereas band No. 1 and 2 were present only in the 0.50% and 0.75% salinity treated plants. The band No.11 was present only under control condition and band No. 17 was present only in the plants growing at 0.25% salinity.

EC 318954: The Native PAGE study in this genotype revealed the presence of 18 distinct protein bands of which band No. 2,4,5,9,12,14,18,19,20 and 21 were present in all saline treated plants and in the plants growing under control condition. The band No, 8, 10, 11 and 21 were present in the plants growing at 0.25%, 0.50% and 0.75% salinity but absent in the plants growing under control condition. Band No. 12, 16 and 17 were present at 0.25% and 0.50% salinity as well as under control condition but absent at higher salinity level i.e. 0.75% salinity, whereas band No.15 appeared only at low salinity i.e. 0.25% and under control condition.

EC 329299: The Native PAGE study in this genotype revealed the presence of 20 distinct protein bands of which band No. 4, 5, 9,10,11,12,13,14,15 and 19 were common to all salinity treatments and control condition. The polypeptide band No. 1,16,17,18, and 21 were present in the plants growing at 0.25%, 0.50% salinity and under control condition. Band No.6 and 20 were present at 0.25% salinity and under control condition whereas higher level of salinity inhibited the appearance of this band. Band No.8 was present at all salinity levels but absent under control condition, whereas band No. 22 was present only at 0.25% and 0.50% salinity level.

EC 4017103: The Native PAGE study in this genotype revealed the presence of 21 polypeptide bands of which band No.5,13,15,17,19,20 and 22 were common to all salinity treatments and control condition whereas band No.1 was present only in the plants growing at 0.25% and control condition. Band No.2 was present only at 0.50% salinity. Band No.3,4,7,8,9.10,11,12 and 14 were salinity specific and appeared in all saline treatments but were absent in control. Band No.16 was present at 0.25%, 0.50% salinity

Table 16. Native protein banding pattern among selected genotypes of Egyptian clover grown in pots

		Band No. and RM value																					
		0.06	0.08	0.1	0.11	0.15	0.25	0.31	0.36	0.46	0.48	0.51	0.53	0.55	0.56	0.58	0.6	0.62	0.65	0.69	0.73	0.74	0.79
Genotype	**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**
Wardan	0.25%		p	p	pp	ppp			p	p	p	p		p	p	p	p	p		ppp	p	p	p
	0.50%		p	p	pp	ppp			p	p	p	p	p	p	p	p	p	p		ppp	p	p	p
	0.75%		p	p	pp	ppp			p	p	p	pppp	p	p	p	p	p	p		ppp	p	p	p
	1%		p	p	pp	ppp			p	p	p	pppp	p	p	p	p	p	p		pp	p	p	p
	Control				pp	ppp	pp		p	p	p	p		p	p	p	p	pp	p	ppp	p	p	p
EC 407709	0.25%		p		pp	ppp	p		p	p	p	p		p	p	p	p	p	p	ppp	p	p	p
	0.50%		p		pp	ppp			p	p	p	p		p	p	p	p	p		ppp	p	p	p
	0.75%		p		pp	ppp			p	p	p	p		p	p	p	p	p	p	ppp	p	p	p
	1%		p	p	pp	ppp	p		p	p	pp	p	p	p	p	p	p	p	p	ppp	p	p	p
	Control				pp	ppp	p		p	p	pp	p	p	p	p	p	p	p	p	ppp	p	p	p
T 45-1	0.25%			p	p	ppp	p		p	p	p	p	p	p	p		pp	p	p	pp		p	p
	0.50%			p	pp	ppp			p	p	p	p	p		p		p		p	p		p	p
	0.75%	p	p	p	pp	ppp			p	p	p	p	p		p		p		p	pp		p	p
	1%	p	p	p	pp	ppp			p	p	p	p	p		p		p		p	p		p	p
	Control			p	pp	ppp			p	p	p	p	p		pp		p	p	p	pp		p	p
ISH 8020B	0.25%			p	pp	ppp			p	p	p		p		pp		pp	p	p	pp		p	p
	0.50%	p	p	p	pp	ppp			p	p	p		p		p		p		p	pp		p	p
	0.75%	p	p	p	pp	ppp			p	p	p		p		p		p		p	pp		p	p
	1%			p	pp	ppp			p	p	p		p		p		p		p	pp		p	p
	Control			p	pp	ppp			p	p	p	p	p		pp		p		p	pp		p	p
EC 318954	0.25%		pp		pp	pppp			p	p	p	p	p	p	p	p	p	pp	pp	ppp	p	p	p
	0.50%		pp		pp	pppp			p	p	p	p	p	p	p		p	pp	pp	ppp	p	p	p
	0.75%		p		ppp	pppp			p	p	p	pp		p	p				pp	pp	p	p	p
	1%																						
	Control		p		pp	ppp				p			p	p	p	p	p	pp	p	ppp	p		p
EC 329299	0.25%	p	pp		ppp	pppp	p		p	p	p	pp	p	p	p	p	p	pp	p	ppp	p	p	p
	0.50%	p	pp		ppp	pppp			p	p	p	pp	p	p	p	p	p	pp	p	ppp		p	p
	0.75%		pp		pp	ppp			p	p	p	p	p	p	p	p				p			
	1%																						
	Control	p	p		ppp	ppp	p			p	p	p	p	p	p	p	p	pp	pp	ppp	p	p	
EC 4017103	0.25%	p		p	ppp	pppp		p	pp	pp	p	pp	p	p	p	p	p	pp		ppp	p		p
	0.50%		p	p	ppp	pppp		p	pp	pp	p	pp	p	p	p	p	p	pp	pp	ppp	p		p
	0.75%			p	ppp	pppp		p	pp	pp	p	pp	p	p	p	p		pp		ppp	p		p
	1%																						
	Control	p				ppp								p		p	p	pp	pp	ppp	p	p	p
T 5-90I-1	0.25%	p		p	pp	pppp			p	p	p	pp		p	p	p	p	p	p	pp	p		p
	0.50%			p	ppp	pppp			p	pp	p	pp		p	p	p	p	p	p	pp	p		p
	0.75%			p	ppp	pppp			p	pp	p	pp		p	p	p		p	p	pp	p		p
	1%																						
	Control	p		p		ppp			p					p	p		p	p	p	p	p		p

and under control condition, whereas band No. 18 was present at 0.50% salinity and control condition. The band No.21 was present only in the plants growing under control condition.

T 5-90I-1: The protein PAGE study revealed the presence of 17 polypeptide bands of which band No.2,5,8,13,14,17,18,19,20 and 21 were common to both treated and non-treatment plants, the band No.1 was present only at low salinity i.e. 0.25% and control plants. Band No. 4,9,10,11 and 15 were salinity specific and were present at all saline treatments but absent in the control plants, whereas band No.16 was present at 0.25%, 0.50% salinity and under control.

D.2. SDS Protein

Sodium dodecylsulphate polyacrylamide (SDS) gel electrophoresis using 10% acrylamide gels were used for high and low molecular weight separation of protein under denatured condition. 27 protein bands were scored for their consistency and clarity. The bands were assigned numbers, starting from the place of origin according to the molecular weight in Kd. The band were identified as band No. 1 (172.7Kd), 2(109Kd), 3 (97.4Kd), 4(90.6Kd), 5(87.2Kd), 6(83.8Kd), 7(73.6Kd), 8(56.8Kd), 9(45.3Kd), 10(43Kd), 11(42.12Kd), 12 (38.62Kd), 13(35.11Kd), 14(34.24Kd), 15(31.61Kd), 16(30.73Kd), 17(27.5Kd) 18(26.75Kd), 19(25.25Kd), 20(24.5Kd), 21(21.5Kd), 22(20.75Kd), 23(17.6Kd), 24(15.2Kd), 25(14Kd), 26(6.5Kd) and 27(3Kd). Observations were recorded for differences in the protein profiles of the 8 selected genotypes of *T. alexandrinum* subjected to 4 levels of saline treatment (0.25%, 0.50%, 0.75% and 1%) and control conditions. The protein profile of the genotypes is presented hereunder (Table 17).

Wardan: The protein profile study of this genotype revealed the presence of 21 distinct polypeptide bands of which band No. 1, 2, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25 and 26 were common to all the saline treatments and in plants growing under control condition, whereas band No.6 and 7 were absent in 0.25% salinity but present in all other salinity level and control plants. The band No. 13 was absent only at 1% salinity.

EC 407709: The protein profile study of this genotype revealed the presence of 21 polypeptide bands of which band No. 1, 2, 8, 9, 10, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 24, 25 and 26 were common to both the treated and non-treated plants whereas band No. 6 and 7 were present only at 0.25% salinity and under control condition.

T 45-1: The protein profile study in this genotype revealed the presence of 23 distinct polypeptide bands of which band No.7, 8, 9, 10, 12, 13, 14, 16, 17, 18, 19, 20, 21, 23, 24, 25, 26 and 27 were common to both treated and non-treated plants whereas band No. 1

and 2 were absent at 0.25% salinity only. The bands No.2 was present at 0.50%, 0.75% and 1% salinity only and band No.4 was present only at low salinity i.e. 0.25% and control condition.

ISH 8020B: The protein profile study in this genotype revealed the presence of 23 polypeptide bands of which band No. 1, 2, 3, 7, 8, 12, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26 and 27 were common to both treated and non-treated plants whereas band No.6 was present at 0.25% salinity and control condition. The Band No.9, 10, 13 and 14 were absent only at 1% salinity level and the band No.19 was present at 0.25%, 0.50% and control condition only.

EC 318954: The SDS gel electrophoresis study of this genotype revealed the presence of 26 polypeptide bands of which band No. 1, 2, 3, 6, 8, 9, 10, 11, 12, 14, 15, 16, 17, 18, 19, 21, 22, 23, 24 and 26 were common to all saline treatments as well as control plants. However, band No. 4 and 5 were present only at 0.25%, 0.50% and control plants. Band No. 7 and 20 were present only at low salinity i.e. 0.25% and control conditions the band No. 13 was absent only at 0.25% salinity level.

EC 329299: The protein profile study in this genotype revealed the presence of 26 polypeptide bands of which band No. 1, 6, 8, 9, 10, 11, 12, 15, 17, 18, 19, 22, 23, 24, 25 and 26 were present at all salinity and under control treatments whereas band No. 2,3,4,5,14,16 and 21 were absent only at 0.75% salinity. Band No.7 was present only at 0.25% salinity and control plants. Band No.13 was present only under control plants and band No. 20 was present only at salinity 0.25% and 0.50%.

EC 4017103: The protein profile study in this genotype revealed the presence of 20 polypeptide bands of which band No. 1,2,8,9,10,12,14,15,16,17,18,20,22,23,25 and 26 were common to plants growing under salinity as well as under control condition, the band No. 13 and 21 were salt specific and present at all treatments but absent under control. Band No. 19 was absent only at 0.25% salinity.

T 5-90I-1: The SDS gel electrophoresis study in this genotype revealed the presence of 20 polypeptide bands of which band No. 1, 8, 9, 10, 12, 14, 15, 17, 18, 22, 23, 25 and 26 were common to both treated and non-treated plants, band No. 2 was present only at low salinity i.e. 0.25% and control condition. Band No. 13, 19, 20 and 21 were present at all salinity levels but absent under control plants, however band No. 16 was present only under control plants.

Table 17. SDS Protein banding patter of selected genotypes of Egyptian clover grown in pots.

		Molecular Weight in Kd and band numbers																										
		172.7	109	97.4	90.6	87.2	83.8	73.6	56.8	45.3	43	42.12	38.62	35.11	34.24	31.61	30.73	27.5	26.75	25.25	24.5	21.5	20.75	17.6	15.2	14	6.5	3
Genotype	**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**	**27**
Wardan	0.25%	pp	p						pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	0.50%	pp	p				p	p	pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	0.75%	pp	p				p	p	pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	1%	pp	p				p	p	pppp	pp	pp		pp		p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	Control	pp	p				p	p	pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
EC 407709	0.25%	pp	p				p	p	pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	0.50%	pp	p						pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	0.75%	pp	p						pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	1%	pp	p						pppp	pp	pp		pp		p	p	p	pp	pp	p	p	p	p		p	p	ppp	
	Control	pp	p				p	p	pppp	pp	pp		pp	p	p	p	p	pp	pp	p	p	p	p		p	p	ppp	
T 45-1	0.25%						p	p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	p	p	pp	ppp	pp
	0.50%	pp	p	p				p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	p	pp	pp	ppp	pp
	0.75%	pp	p	p				p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	p	pp	pp	ppp	pp
	1%	pp	p	p				p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	pp	pp	pp	ppp	pp
	Control	pp		p			p	p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	p	p	pp	ppp	pp
ISH 8020B	0.25%	pp	p	p			p	p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	p	pp	pp	ppp	pp
	0.50%	pp	p	p				p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	pp	pp	pp	ppp	pp
	0.75%	pp	p	p				p	pppp	p	p		ppp	p	p		pp	p	p		p	p	p	p	pp	pp	ppp	pp
	1%	pp	p	p				p	ppp				pp				p	p	p		p	p	p	p	p	pp	pp	pp
	Control	pp	p	p			p	p	pppp	p	p		ppp	p	p		pp	p	p	p	p	p	p	p	pp	pp	ppp	pp
EC 318954	0.25%	pp	p	p	p	p	p	p	pppp	pp	pp	p	ppp		p	p	p	pp	pp	p	p	p	p	p	pp	pp	ppp	
	0.50%	pp	p	p	p	p	p		pppp	pp	pp	p	ppp	p	p	p	p	pp	pp	p		p	p	p	pp	pp	ppp	
	0.75%	pp	p	p			p		pppp	pp	pp	p	ppp	p	p	p	p	pp	pp	p		p	p	p	pp	pp	pp	
	1%																											
	Control	pp	p	p	p	p	p	p	pppp	pp	pp	p	ppp	p	p	p	p	pp	pp	p	p	p	p	p	pp	pp	pp	
EC 329299	0.25%	pp	p	p	p	p	p	p	pppp	pp	pp	p	ppp		pp	pp	p	pp	pp	p	p	p	p	pp	pp	pp	ppp	
	0.50%	pp	p	p	p	p	p		pppp	pp	pp	p	ppp		pp	pp	p	pp	pp	p	pp	p	p	pp	pp	pp	ppp	
	0.75%	p					p		pp	p	p	p	ppp			p		p	p	p			p	p	p	p	pp	
	1%																											
	Control	pp	p	p	p	p	p	p	pppp	pp	pp	p	ppp	p	p	pp	p	pp	pp	pp		p	p	pp	pp	pp	ppp	
EC 4017103	0.25%	pp	p						pp	p	p		pp	p	p	p	p	p	p		p	p	p	p	p	pp	ppp	
	0.50%	pp	p						ppp	p	p		p	p	p	p	p	p	p	p	p	p	p	p	p	pp	pp	
	0.75%	pp	p						pppp	p	p		pp	p	p	p	p	p	pp	p	p	p	p	pp	pp	pp	ppp	
	1%																											
	Control	pp	p						ppp	p	p		pp		p	p	p	p	p	p	p		p	p	p	pp	ppp	
T 5-90I-1	0.25%	pp	p						pp	p	p		pp	p	p	p		p	p	p	p	p	p	p	p	p	pp	
	0.50%	pp							pp	p	p		p	p	p	p		p	p	p	p	p	p	p	p	pp	p	
	0.75%	pp							pp	p	p		p	p	p	p		p	p	p	p	p	p	p	p	pp	p	
	1%															p												
	Control	pp	p						pp	p	p		pp		p	p	p	p	p				p	p	p	pp	pp	

D.3. Sodium and Potassium ion estimation

The Na^+ and K^+ ions were estimated in the 8 selected genotypes of *T. alexandrinum* growing at 4 salinity treatments (0.25%, 0.50%, 0.75% and 1% NaCl) and control condition by Digital Flame Photometer using the method given by G.H. Jeffery et al. (1989). The results are presented in Table (18 and 19).

Wardan: Na^+ content in the roots increased gradually with increasing salinity levels as compared to control, it was highest at 0.75% salinity level. The K^+ content declined marginally at 0.25% and 0.50% salinity level whereas at 0.75% and 1% salinity it increased as compared to control. The K^+ content was more as compared to Na^+ at all salinity levels. The Na^+: K^+ ratio increased at all salinity levels as compared to control.

The Na^+ content in the shoot region declined under saline conditions except at 1% salinity level as compared to control. The K^+ content also declined under salinity treatment except at 1% salinity as compared to control. The Na^+: K^+ ratio was reduced under saline conditions except at 1% salinity as compared to the plants growing under normal condition.

EC 407709: The Na^+ content in the roots of this genotype increased at increasing salinity levels as compared to control. The K^+ content decreased under saline conditions as compared to control. The Na^+: K^+ ratio in the roots in stress condition was almost twice as compared to control.

The Na^+ content in the shoot region increased with increasing salinity level and was almost twice at higher salinity as compared to control. The K^+ content in shoot was reduced at 0.25% salinity whereas at higher salinity i.e. 0.50% and 0.75% salinity it increased as compared to control. The Na^+: K^+ ratio increased under saline conditions as compared to control.

T 45-1: The Na^+ content in the roots increased at all salinity levels as compared to control. The K^+ content declined at all salinity levels as compared to control. The Na^+ : K^+ ratio in the root portion gradually increased with increasing salinity level as compared to control.

The Na^+ content in the shoot region increased under saline conditions as compared to control. The K^+ content in the shoot region declined very marginally at 0.25% and 0.50% salinity whereas at 0.75% the reduction was more pronounced as compared to control. The Na^+ : K^+ ratio in the shoot region increased gradually with increasing salinity level as compared to control.

EC 318954: The Na^+ content in the root portion increased gradually with increased salinity level as compared to control. The K^+ content in root region increased marginally (452mM/gdw) at 0.25% salinity but at higher salinity i.e. at 0.50% and 0.75% it declined as compared to control (405 mM/gDW). The Na^+: K^+ ratio in the root region declined very marginally at 0.25% salinity but increased with increased salinity level as compared to control.

The Na^+ content in the shoot region declined very marginally at 0.25% salinity whereas at 0.50% and 0.75% salinity it increased as compared to control. The K^+ content in the shoot region declined under saline conditions as compared to control. The Na^+: K^+ ratio in the shoot region increased under saline conditions as compared to control.

EC 329299: The Na^+ content in the root region declined at 0.25% salinity whereas at 0.50% it marginally increased and than again decreased at 0.75% salinity as compared to control. The K^+ content in the root region decreased under saline conditions as compared to the control. The Na^+: K^+ ratio declined at 0.25% and 0.75% salinity whereas at 0.50% salinity it increased as compared to control.

The Na^+ content in the shoot region reduced under saline conditions as compared to control. The K^+ content also decreased under salinity treatments as compared to control. The Na^+: K^+ ratio in the shoot region declined at 0.25% salinity whereas at 0.50% and 0.75% salinity it increased as compared to control.

EC 4017103: The Na^+ content in the roots increased with increasing salinity as compared to control. The K^+ content decreased under saline conditions as compared to control. The Na^+ : K^+ ratio in the roots increased with increasing salinity level as compared to control.

The Na^+ content in the shoot region increased under saline conditions as compared to control. The K^+ ion content decreased at 0.25% salinity whereas at 0.50% and 0.75% salinity it increased as compared to control. The Na^+ : K^+ ratio increased under saline conditions as compared to control.

T 5-90I-1: The Na^+ content in the root region declined at 0.25% salinity whereas at 0.50% salinity it was estimated to be more than control. The K^+ content also had a similar trend of decline at 0.25% and increased presence at 0.50% salinity as compared to control. The Na^+ : K^+ ratio increased marginally at saline conditions as compared to control.

The Na^+ content in the shoot region increased proportionally with increasing salinity as compared to control. The K^+ content increased with increasing salinity as compared to control plants. The Na^+ : K^+ ratio increased with increasing salinity as compared to control plants.

Table 18. Na+ and K+ ion estimation in roots of selected genotypes of Egyptian clover grown in pots						
		Na+ concentration		K+ concentration		
Genotype	Treatment	%	M mol/g	%	M mol/g	Na+ : K+
Wardan	0.25%	0.8375	360.86	0.85	447.36	0.8
	0.50%	1.25	543.47	0.775	407.89	1.33
	0.75%	1.2625	547.82	0.9875	515.78	1.06
	1%	0.9375	404.34	1.1875	621.05	0.65
	Control	0.625	269.56	0.9375	489.47	0.55
EC 407709	0.50%	1.25	543.47	0.7875	410.52	1.32
	0.75%	1.375	578.26	1.05	552.89	1.04
	Control	0.875	378.26	1.475	757.89	0.49
T 45-1	0.25%	1.15	500	1.15	605.26	0.82
	0.50%	0.75	326.08	0.925	484.21	0.67
	0.75%	1.2375	534.78	0.9875	515.78	1.03
	Control	0.7125	308.69	1.3125	689.47	0.44
EC 318954	0.25%	0.8875	382.6	0.8625	452.63	0.84
	0.50%	1.125	486.95	0.625	326.31	1.49
	0.75%	0.9875	426.08	0.45	236.84	1.79
	Control	0.85	369.56	0.775	405.26	0.91
EC 329299	0.25%	0.7625	330.43	1.1125	584.21	0.56
	0.50%	1.1625	504.34	0.9625	505.26	0.99
	0.75%	1.05	456.52	1.25	657.89	0.69
	Control	1.1	478.26	1.2875	673.68	0.7
EC 4017103	0.25%	0.825	356.52	1.4125	742.1	0.48
	0.50%	1.2375	534.78	1.3375	700	0.76
	0.75%	1.175	508.69	1.65	868.42	0.58
	Control	0.8375	360.86	1.875	948.21	0.36
T 5-90I-1	0.25%	0.6375	273.91	1.2125	636.84	0.43
	0.50%	1.1376	491.3	1.6625	873.68	0.56
	Control	0.775	334.78	1.5125	794.73	0.42
ISH 8020B	0.25%	0.6875	400	1.2625	721.05	0.55
	0.50%	1.0875	482.6	1.4	615.78	0.78
	0.75%	1.4875	417.39	1.5625	578.94	0.72
	Control	0.875	282.6	1.05	894.7	0.31

Table 19. Na+ and K+ ion estimation in shoots of selected genotypes of Egyptian clover grown in pots						
		Na+ Concentration		K+ concentration		
Genotype	Treatment	%	M mol/g	%	M mol/g	Na+ : K+
Wardan	0.25%	0.7875	339.13	1.025	536.84	0.63
	0.50%	1.125	489.13	1.125	592.1	0.82
	0.75%	1.0125	439.13	0.925	484.4	0.9
	1%	1.6125	700	1.175	615.78	1.13
	Control	1.225	530.43	1.1375	594.73	0.89
EC 407709	0.25%	1.1375	491.3	1.075	563.15	0.87
	0.50%	1.775	769.56	1.25	657.89	1.16
	0.75%	1.5875	686.95	1.4	736.84	0.93
	Control	0.7125	308.69	1.2625	663.15	0.46
T 45-1	0.25%	1.3625	591.3	1.25	657.89	0.89
	0.50%	1.55	673.91	1.1625	610.52	1.1
	0.75%	1.4875	643.47	0.9125	478.94	1.34
	Control	0.8875	382.6	1.2625	663.15	0.57
EC 318954	0.25%	0.975	421.73	0.7375	384.21	1.09
	0.50%	1.125	589.47	1.0125	531.57	1.1
	0.75%	1.2125	526.08	1.1125	584.21	0.9
	Control	1.075	465.21	1.15	605.26	0.76
EC 329299	0.25%	1.0625	460.86	1.2375	647.36	0.71
	0.50%	1.2625	547.82	0.925	484.21	1.13
	0.75%	1.425	617.39	1.2375	647.36	0.95
	Control	1.625	704.34	1.45	763.15	0.92
EC 4017103	0.25%	1.25	657.89	1.1125	584.21	1.12
	0.50%	1.1625	504.34	1.65	868.42	0.58
	0.75%	1.625	704.34	1.4375	752.63	0.93
	Control	1.175	508.69	1.4	736.84	0.69
T 5-90I-1	0.25%	0.9125	395.65	1.575	826.31	0.47
	0.50%	1.4875	643.47	1.5625	821.05	0.78
	Control	0.6875	295.65	1.2625	663.15	0.44
ISH 8020B	0.25%	0.925	469.56	1.375	736.84	0.63
	0.50%	1.1125	643.47	1.175	821.05	0.78
	0.75%	0.9625	378.26	1.1	552.63	0.68
	Control	1.025	443.47	1.2125	636.84	0.69

ISH 8020B: The Na^+ content in the roots of plants growing under saline conditions was higher as compared to control. The K^+ content decreased proportionally to increasing salinity levels as compared to control. The Na^+: K^+ ratio in the roots increased under saline condition as compared to control plants.

The Na^+ content in the shoot region of the plants growing at different salinity treatments had an irregular trend. The amount of Na^+ at 0.25% and 0.50% salinity increased whereas at 0.75% salinity it decreased in comparison to control. The K^+ content in the plants growing at 0.25% and 0.50% salinity increased whereas at 0.75% salinity it was reduced as compared to control. The Na^+: K^+ ratio overall showed irregular pattern but values were more or less equal to control plants.

E. Effect of secondary salinization on selected genotypes in sand culture

Five selected genotypes of Berseem i.e., EC 318954, EC 329299, T 45-1, EC 407709 and ISH 8020B were used. Seeds were germinated in pots containing washed sand moistened with water up to first leaf emergence (10 to 12 days after sowing); the pots were thereafter irrigated with nutrient solutions as given by (Shannon and Noble, 1995) supplemented with 0.50%, 0.75% and 1% NaCl. Seedlings were irrigated every day with 500 ml nutrient solution/pot. The sand of each pots washed every 5th day by irrigating it with running plain water for 5 minutes to prevent salt-build up. The morphological data was recorded on 60th day after sowing. Isozymic and protein banding patter was observed in the leaves collected on 60th day.

E.1. Morphological data

Morphological data recorded on 60th day of growth is described hereunder and presented in Table 20.

ISH 8020B

Plant height: The average height of the plants under control condition was 33.9 cm which increased to 38.8 cm at 0.50% salinity whereas at 0.75% and 1% salinity it decreased to 25.2 cm and 12.2 cm respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 12.1, which increased to 18.1 at 0.50% salinity whereas at 0.75% and 1% salinity it decreased to 9.6 and 6.0 respectively.

Leaf length: The average length of middle leaflet in the plants growing under control condition was 4.0 cm whereas at 0.50%, 0.75% and 1% salinity it was 4.1, 3.8 and 1.8 cm respectively.

Leaf width: The average width of middle leaflet in the plants growing under control condition was 1.0 cm which was 1.1, 0.9 and 0.7 cm at 0.50%, 0.75% and 1% salinity respectively.

Biomass of root: The average biomass of the roots in the plants growing under control condition was 0.72 g whereas at 0.50%, 0.75% and 1% salinity it was 0.77g, 0.50 g and 0.17g respectively.

Biomass of shoot: The average biomass of the shoot in the plants growing under control condition was 3.1 g, which increased at 0.50% to 5.4 g whereas at 0.75% and 0.75% it was 2.14 and 0.61 respectively.

EC 407709

Plant height: The height of the plants growing under control condition was 44.8 cm, which decreased gradually to 37.1, 31.0 and 23.3 cm at 0.50%, 0.75% and 1% salinity respectively.

No. of leaves: The average number of leaves in the plants growing under control condition was 15.3 which reduced to 14.9, 11.1 and 8.7 at 0.50%, 0.75% and 1% salinity respectively.

Leaf length: The average length of the leaves in plants growing under control condition was 4.1, which reduced to 3.3, 3.2 and 2.6 cm at 0.50%, 0.75% and 1% salinity respectively.

Leaf width: The average width of the leaves growing under control condition was 1.2 cm whereas at 0.50%, 0.75% and 1% salinity it was 1.1, 0.9 and 0.9 cm respectively.

Biomass of root: The average biomass of the roots in the plants growing under control condition was 0.67 g whereas at 0.50%, 0.75% and 1% salinity it was reduced to 0.44, 0.43 and 0.21 g respectively.

Biomass of shoot: The average biomass of the shoot portion in the plants growing under control condition was 4.17 g, which was reduced to 2.63, 2.19 and 1.31 g at 0.50%, 0.75% and 1% respectively.

T 45-1

Plant height: The average height of the plants growing under control condition was 43.2 cm, which was reduced to 33.7, 25.9 and 17.5 cm at 0.50%, 0.75% and 1% respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 19.7 whereas at 0.50%, 0.75% and 1% salinity it reduced to 13.1, 9.2 and 6.6 respectively.

Leaf length: The average length of the leaves in the plants growing under control condition was 3.9 cm which gradually decreased to 3.1, 2.5 and 1.9 cm at 0.50%, 0.75% and 1% salinity respectively.

Leaf width: The average width of the leaves in the plants growing under control condition was 1.3 cm, which was reduced to 1.2, 0.9 and 0.9 cm at 0.50%, 0.75% and 1% salinity respectively.

Biomass of root: The average biomass of the roots in the plants growing under control condition was 0.95 g, which was reduced to 0.51, 0.21 and 0.21 g at 0.50%, 0.75% and 1% salinity respectively.

Biomass of shoot: The average biomass of the shoot portion in the plants growing under control condition was 6.20 g, which was reduced to 3.65, 1.76 and 0.89g at 0.50%, 0.75% and 1% salinity treatments respectively.

EC 318954

Plant height: The average height of the plants growing under control condition was 35.2 cm, which was reduced to 25.8, 19.2 and 16.1 cm at 0.50%, 0.75% and 1% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 14.7, which were reduced to 11.4, 9.4 and 8.0 at 0.50%, 0.75% and 1% salinity respectively.

Leaf length: The average length of the leaves in the plants growing under control condition was 3.8 cm, which gradually reduced with increasing salinity to 2.7, 2.1 and 1.8 cm at 0.50%, 0.75% and 1% salinity respectively.

Leaf width: The average width of the leaves in the plants growing under control condition was 1.2 cm which was reduced to 1.0, 0.8 and 0.7 cm at 0.50%, 0.75% and 1% salinity respectively.

Biomass of root: The average biomass of the roots in the plants growing under control condition was 0.48 g which decreased proportionally to increased salinity treatment. At salinity 0.50%, 0.75% and 1% it was reduced to 0.35g, 0.32 g and 0.14g respectively.

Biomass of shoot: The average biomass of the shoot portion in the plants growing under control condition was 4.11g which was reduced to 2.21g, 1.36g and 1.02g at 0.50%, 0.75% and 1% salinity respectively.

EC 329299

Plant height: The average height of the plants growing under control condition was 33.2 cm which was reduced to 26.7, 18.8 and 13.8 cm at 0.50%, 0.75% and 1% salinity respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 12.8 which were reduced to 10.8, 8.0, and 5.9 at 0.50%, 0.75% and 1% salinity respectively.

Leaf length: The average length of leaves in the plants growing under control condition was 3.5 cm which was reduced to 2.8, 2.2 and 1.8 cm at 0.50%, 0.75% and 1% salinity respectively

Leaf width: The average length of leaves in the plants growing under control condition was 1.2 cm which was reduced to 1.0, 0.8 and 0.7 cm at 0.50%, 0.75% and 1% salinity respectively.

Biomass of root: The average biomass of the roots in the plants growing under control condition was 0.70g which was reduced to 0.36, 0.31 and 0.17g at 0.50%, 0.75% and 1% salinity respectively.

Biomass of shoot: The average biomass of the shoot portion in the plants growing under control condition was 3.36 g that reduced to 2.25, 1.20 and 0.68g at 0.50%, 0.75% and 1% salinity respectively.

Analysis of variance for all the traits observed revealed significant differences both at genotypic level as well as at different salinity levels (Table 21).

E.2. Isozyme analysis

Isozyme studies were conducted using vertical PAGE gel electrophoresis to study the variations for the isozymic pattern of 2 enzymes i.e., SOD (Super Oxide dismutase) and Est. (Esterase) under 3 different levels of salinity (0.50%, 0.75% and 1%) compared with seedling growing under control condition. The unambiguous bands were scored and given numbers starting from the place of origin. Bands were scored across the plates and genotypes (Table 22).

E.2.1 Banding pattern of different enzymes

Super Oxide Dismutase (SOD): The study revealed the presence of 4 bands identified as band No.1, (RM 0.21), 2 (RM 0.22), 3 (RM 0.33) and 4 (RM 0.46) in saline as well as control condition.

Esterase: Esterase isozymic banding pattern revealed the presence of 11 bands, which were, identified as band No. 1 (RM 0.15), 2 (RM 0.24), 3 (RM 0.30), 4 (RM 0.32), 5 (RM

Table 20. Morphological parameters and biomass of (60 day old plants) of Egyptian clover genotypes growing under sand culture conditions.

Genotype	Treatment	Germination (%)	Plant Height (cm)	No. of leaves	Leaf Length (cm)	Leaf width	Weight of plant (g)	Weight of root (g)	Weight of shoot (g)
ISH 8020B	0.50%	70.0	38.8	18.1	4.1	1.1	6.17	0.77	5.40
	0.75%	65.0	25.2	9.6	3.8	0.9	2.64	0.50	2.14
	1%	68.3	12.2	6.0	1.8	0.7	0.78	0.17	0.61
	Control	81.7	33.9	12.1	4.0	1.0	3.82	0.72	3.10
EC 407709	0.50%	78.3	37.1	14.9	3.3	1.1	3.07	0.44	2.63
	0.75%	68.3	31.0	11.1	3.2	0.9	2.63	0.43	2.19
	1%	71.7	23.3	8.7	2.6	0.9	1.52	0.21	1.31
	Control	90.0	44.8	15.3	4.1	1.2	4.84	0.67	4.17
T 45-1	0.50%	80.0	33.7	13.1	3.1	1.2	4.16	0.51	3.65
	0.75%	73.3	25.9	9.2	2.5	0.9	1.98	0.21	1.76
	1%	70.0	17.5	6.6	1.9	0.9	1.10	0.21	0.89
	Control	81.7	43.2	19.7	3.9	1.3	7.15	0.95	6.20
EC 318954	0.50%	76.7	25.8	11.4	2.7	1.0	2.55	0.35	2.21
	0.75%	71.7	19.2	9.4	2.1	0.8	1.68	0.32	1.36
	1%	75.0	16.1	8.0	1.8	0.7	1.16	0.14	1.02
	Control	78.3	35.2	14.7	3.8	1.2	4.59	0.48	4.11
EC 329299	0.50%	81.7	26.7	10.8	2.8	1.0	2.61	0.36	2.25
	0.75%	70.0	18.8	8.0	2.2	0.8	1.51	0.31	1.20
	1%	75.0	13.8	5.9	1.8	0.7	0.85	0.17	0.68
	Control	83.3	33.2	12.8	3.5	1.2	4.06	0.70	3.36

Table 21. Analysis of variance (Two factor) for morphological parameters and biomass in 60 day old plants of Egyptian clover genotypes.			
Source of Variation	*SS*	*df*	*MS*
Plant height			
Sample	959.234	4	239.809**
Columns	3987.725	3	1329.242**
Interaction	357.198	12	29.767*
No. of leaves			
Sample	73.049	4	18.262*
Columns	604.161	3	201.387**
Interaction	170.495	12	14.208*
Leaf length			
Sample	7.632	4	1.908**
Columns	27.660	3	9.22**
Interaction	4.266	12	0.355**
Leaf width			
Sample	0.152	4	0.038**
Columns	1.501	3	0.5**
Interaction	0.222	12	0.018*
weight of plant			
Sample	15.257	4	3.814**
Columns	128.806	3	42.935**
Interaction	37.831	12	3.153**
weight of root			
Sample	0.331	4	0.083**
Columns	2.185	3	0.728**
Interaction	0.527	12	0.044*
weight of shoot			
Sample	11.971	4	2.993**
Columns	97.883	3	32.628**
Interaction	30.889	12	2.574**

0.33), 6 (RM 0.40), 7 (RM 0.44), 8 (RM 0.47), 9 (RM 0.49), 10 (RM 0.53) and 11 (RM 0.54) in saline as well as control.

EC 329299

SOD: In this genotype 4 distinct bands were resolved. The entire 4 band i.e., band No.1, 2, 3 and 4 were present in the treated as well as under non-treatment plants.

Esterase: Esterase isozymic study revealed the presence of 9 bands of which band No.3, 8 and 9 were common to both treated and non-treated plants. Band No.1 was present only at 0.50% salinity. Band No.2 and 11 were present only under control condition. Band No.5 and 7 were present at all salinity treatments but absent under control condition whereas band No. 10 was present only in 1% salinity treatment.

EC 318954

SOD: In this genotypes 4 bands were resolved i.e., band No. 1, 2, 3 and 4 and all the 4 bands were common to both treated and non-treatment plants.

Esterase: Esterase isozymic study revealed the presence of 7 distinct bands of which band No.3, 7, 8 and 9 were common to both treatment and non-treatment plants. Band No. 4 was present only in 1% salinity treated plants. Band No.5 and 10 were present in 0.50%, 1% and control plants but absent at 0.75% salinity treatment.

T 45-1

SOD: In this genotypes the SOD banding pattern revealed the presence of 4 distinct bands of which band No. 1, 3 and 4 were common to both treated and non-treatment plants whereas Band No. 2 was present only in 0.50% and 0.75% salinity treatments only.

Esterase: Esterase isozymic banding pattern revealed the presence of 6 distinct bands of which band No.3, 5, 7, 8 and 9 were present in both the treatment and non-treated plants whereas band No.4 was present only in 0.50% and 1% salinity treatment plants.

EC 407709

SOD: SOD banding pattern revealed the presence of 4 distinct bands i.e., band No.1, 2, 3 and 4 of which band No.2 was present only in 1% treated plants and absent in other salinity treatments and control plants.

Esterase: Esterase isozymic banding pattern revealed the presence of 7 distinct bands of which band No. 3, 7, 8, 9 and 10 were common to both treated and non-treated plants. Band No.1 was present only 0.50% treated plants whereas band No.5 was present only in 0.75% and control plants only.

Table 22. Isozyme banding pattern of (60 days old plants) of Egyptian clover genotypes growing in sand culture conditions.

		Esterase											S O D			
		Band No and RM value														
		0.15	0.24	0.30	0.32	0.33	0.40	0.44	0.47	0.49	0.53	0.54	0.21	0.22	0.33	0.46
Genotype	Treatmen	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4
EC 329299	0.50%	p		ppp		p		p	ppp	pppp			ppp		ppp	ppp
	0.75%			ppp		p		p	ppp	pppp			ppp	pp	ppp	pppp
	1%			pp		p		p	p	p	p		ppp	ppp	ppp	pp
	Control		p	pp					p	p		p	pp	p	pppp	pppp
EC 318954	0.50%			ppp		p		pp	ppp	pppp	pp		ppp	pp	pp	pp
	0.75%			ppp				p	pp	pp			ppp	pp	ppp	ppp
	1%			pp	p	p		pp	ppp	ppp	p		ppp	p	ppp	ppp
	Control			pp		p		p	pp	pp	p		ppp	p	pppp	pppp
T 45-1	0.50%			ppp	p	p		p	ppp	ppp			ppp	pp	pp	pp
	0.75%			pp		p		p	pp	pp			ppp	pp	pppp	pppp
	1%			pp	p	p		p	pp	pp			pp		pppp	ppp
	Control			pp		p		p	pp	ppp			pp		ppp	ppp
EC 407709	0.50%	p		ppp				pp	ppp	pppp	p		ppp		p	pp
	0.75%			pp		p		p	pp	ppp	p		ppp		pp	ppp
	1%			pp				p	pp	ppp	p		ppp	p	pp	ppp
	Control			pp		p		p	pp	ppp	p		pp		pp	ppp
ISH 8020B	0.50%			ppp				p					ppp	ppp	pp	ppp
	0.75%			pp				p					pp	pp	p	ppp
	1%			ppp			p	pp	ppp	ppp	p		pp		pp	ppp
	Control			pp		p		p	pp	ppp			pp		p	pp

ISH 8020B

SOD: SOD banding pattern revealed the presence of 4 distinct bands of which band No. 1, 3, and 4 were common to both treated and non-treated plants whereas band No.2 was present only in 0.50% and 0.75% salinity only.

Esterase: Esterase banding pattern revealed the presence of 7 distinct bands of which band No.3 and 7 was common to both treated and non-treated plants Band No.5 was present only in control plants, band No.6 and 10 was present only in 1% treated plants whereas band No.8 and 9 were present only in 1% and control plants.

E.3. Native protein

The electrophoratic banding pattern of the plant samples collected from 60 day old plants grown in pots filled with sand and treated with 3 different salinity treatments i.e., 0.50%, 0.75% and 1% NaCl and without NaCl i.e., control condition was carried out using Native-PAGE. The study revealed the presence of 17 bands. The bands were scored and given numbers based on relative mobility staring from the place of origin. The bands were identified as band No.1 (RM 0.13), 2 (RM 0.16), 3 (RM 0.19), 4 (RM 0.20), 5 (RM 0.24), 6 (RM 0.26), 7 (RM 0.35), 8 (RM 0.42), 9 (RM 0.45), 10 (RM 0.48), 11 (RM 0.51), 12 (RM 0.54), 13 (RM 0.57), 14 (RM 0.61), 15 (RM 0.64), 16 (RM 0.69), and 17(RM 0.71) (Table 23).

EC 329299: The banding pattern revealed the presence of 14 distinct bands of which band No. 1, 2, 3, 6, 7, 10, 12, 13, 15 and 17 were common to both treated and non-treatment plants. Band No.9 was present in 0.50% and 1% salinity treatment plants only, whereas band No.11 was present in 0.50%, 0.75% and control plants. Band No. 14 was present in plants growing at 0.75%, 1% salinity and under control conditions, whereas band No.16 was present only in plants growing at 0.75% and 1% salinity only.

EC 318954: The banding pattern revealed the presence of 16 bands of which band No. 2, 3, 6, 7, 10, 12, 13, 15 and 17 was common to both treated and non-treated plants. Band No.1 and 9 was present in 0.50% and 0.75% salinity treated plants and under control condition. Band No.4 was present only in plant growing in 0.75% salinity treatment whereas band No.5 was present only in plants growing under control condition. Band No.9 was present in 0.50% and 0.75% salinity treatments and under control condition. Band No. 11 was present in plants growing at 0.50%, 1% salinity and in plants growing under control condition. Band No.14 was present in plants growing at 0.75% and 1% salinity as well as in plants growing under control condition. Band No.16 was present at 0.50%, 0.75% and 1% salinity treatment but absent under control condition.

Table 23. Native protein banding pattern in (60 days old plants) of Egyptian clover genotypes in sand culture condition.

		Band No. and RM value																
		0.13	0.16	0.19	0.2	0.24	0.26	0.35	0.42	0.45	0.48	0.51	0.54	0.57	0.61	0.64	0.69	0.71
Genotype	**Treatment**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**16**	**17**
EC 329299	0.50%	p	pp	ppp			p	p		p	p	p	p	p		p		p
	0.75%	p	pp	ppp			p	p			pp	p	pp	pp	p	pp	p	p
	1%	p	pp	p			p	p		pp	p		pp	ppp	p	ppp	p	p
	Control	p	p	ppp			p	p			p	p	p	pp	p	pp		pp
EC 318954	0.50%	p	p	ppp			p	p		p	pp	p	p	pp		pp	p	p
	0.75%	p	p	ppp	p		p	p		p	p		p	pp	p	pp	p	p
	1%		pp	ppp			p	p			pp	p	p	pp	p	pp	p	p
	Control	p	pp	ppp		p	p	p		p	p	p	p	pp	p	pp		p
T 45-1	0.50%		pp	ppp		p	p	p		p	p	p	p	pp		p	p	p
	0.75%		pp	ppp		p	p	pp		p	pp	p	pp	ppp	pp	pp	p	p
	1%	p	pp	ppp			p	p	p	p	p	p	pp	ppp	pp	pp	p	p
	Control		pp	ppp			p	pp	p	p	p	p	pp	pp	p	p		p
EC 407709	0.50%	p	pp	ppp		p	p	pp	p	p	ppp		ppp	ppp		ppp	p	p
	0.75%	p	pp	ppp		p	p	p	p	p	pp		pp	pp	p	pp	p	p
	1%	p	pp	pp		p	p	p		p	ppp		ppp	ppp	p	ppp	p	p
	Control	p	pp	ppp			p	p		p	pp		pp	pp	p	p	p	p
ISH 8020B	0.50%	p	p	pp	ppp		p	p	p	p	pp		ppp	ppp	p	pp	pp	p
	0.75%	p	p	pp	ppp		p	p		p	pp		ppp	ppp	p	ppp	pp	p
	1%		pp	ppp		p	p	p		p	pp		ppp	ppp	p	ppp	p	p
	Control		pp	ppp		p	p	p		p	pp		pp	pp		pp	p	pp

T 45-1: In this genotypes 16 polypeptide bands were resolved across the plate of which band No.2, 3, 6, 7, 9, 10, 11, 12, 13, 15 and 17 were common to both treated and non-treated plants whereas band No.1 was present in 1% salinity treated plants only. Band No.5 was present only in 0.50% and 0.75% salinity treatments. Band No.8 was present only in 1% salinity treatment and in control plants. Band No. 14 was present in 0.75%, 1% salinity treated plants and in plants growing in 0.50%, 0.75% and 1% salinity treated plants only.

EC 407709: The native PAGE gel electrophoresis revealed the presence of 15 polypeptide distinct bands of which band No. 1, 2, 3, 6, 7, 9, 10, 12, 13, 13, 15, 16 and 17 were common to both treated and non-treated plants whereas band No.5 was present in all the salinity treatment but absent under control condition. Band No.8 was present in 0.50% and 0.75% salinity treatments only. Band No. 14 was present in 0.75%, 1% salinity treatment and in plants growing under control condition.

ISH 8020B : The banding pattern in this genotype revealed the presence of 16 distinct bands of which band No. 2, 3, 6, 7, 9, 10, 12, 13, 15, 16 and 17 were common to both treated and non-treated plants, whereas band No.1 and 4 were present only in 0.50% and 0.75% salinity treatments. Band No.5 was present only in plants growing at 1% salinity and under control condition. Band No.8 was present only in plants growing at 0.50% salinity, whereas band No.14 was present at all salinity treatment but absent in control.

E.4. SDS PAGE Protein banding pattern

Sodium dodecylsulphate polyacrylamide (SDS) gel electrophoresis using 10% acrylamide gels were used for high and low molecular weight separation of protein under denature condition. 23 protein bands ranging between 205 Kd to 20 Kd were scored in the 5 different genotypes of *Trifolium alexandrinum* subjected to 3 levels of salinity treatments (0.50%, 0.75%, and 1%) and control condition. The protein profile revealed that 11 bands were monomorphic (Table 24). Polymorphism was observed for 12 bands (band no. 1, 3, 4, 5, 8, 9, 10, 16, 17, 19, 20, 21) causing genetic variation in 5 genotypes.

EC 329299: The protein profile study of this genotype revealed the presence of 23 distinct bands of which band No. 2, 3, 4, 5, 6, 7, 9, 10, 11, 12, 13, 14, 15, 17, 18, 21, 22 and 23 were common to all saline treatments and in plants growing under control condition whereas band No.1 was absent in 0.50% and 0.75% salinity. Band No.8 was absent only at 0.75% salinity, band No.16, 19 and 20 was absent under control condition only.

EC 318954: The protein profile of this genotype revealed the presence of 23 distinct polypeptide bands of which band No. 1, 2, 5, 6, 7, 8, 10, 11, 12, 13, 14, 15, 18, 19, 22 and 23 were common to all saline treatments as well as under control condition whereas band No. 3 was absent only at 0.50% salinity level. Band No.4 was present only at 0.50% salinity and band No.9, 16, 17, 20 and 21 were absent under control condition

T 45-1: The protein profile study in this study revealed the presence of 23 distinct polypeptide bands of which band No. 1, 2, 3, 5, 7, 11, 12, 13, 14, 15, 17, 18, 20, 21, 22 and 23 were common to all saline treatments as well as under control condition whereas band No. 4 was absent at 0.50% and 0.75% salinity. Band No. 8 and 19 were absent under control condition, band No. 9 and 10 were absent at 1% salinity condition and band No. 16 was absent at 0.50% salinity and control condition only.

EC 407709: The protein profile study in this genotype revealed the presence of 23 distinct protein bands of which band No. 2, 3, 4, 5, 7, 8, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22 and 23 were common to all saline treatments as well as control condition whereas band No. 1 was absent only at 1% salinity. Band No. 5 was absent only at 0.75% salinity and band No. 9 was absent at 0.50% and 0.75% salinity.

ISH 8020B: The protein profile study in this genotype revealed the presence of 23 distinct protein bands of which band No. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, 20, 21, 22 and 23 were common to all saline treatments as well as control condition whereas band No. 16 was absent under control condition.

F. *In vitro* callusing response under saline vis-à-vis normal condition.

In vitro response of various explants to varying levels of salinity in callus inducing media L_2 were carried out in 3 different genotypes of *T. alexandrinum* i.e., Wardan, EC 318954 and EC 329299. Experiment was carried out to study the interaction effect of various factors such as genotype, explants source, media, levels of salinity etc. The various parameters taken into consideration for evaluation include percent callus induction and nature, growth, colour and size of the callus.

Healthy and viable seeds of the three genotypes were selected and allowed to germinate *in vitro* on MS media devoid of any hormones, under aseptic culture conditions. The seeds germinated within 3-5 days of culture. The seeds developed into 3–4 cm long seedlings by 20th day and at this stage the explants hypocotyls and petiole were excised and cultured on media containing 4 different salinity levels i.e., 0.25%, 0.50%, 0.75% in

Table 24. SDS Protein profile in 60 day old plants of Egyptian clover genotypes growing in sand culture condition.

		Band Numbers / MW in Kd																						
Genotype	Treatment	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
		205	173	97.4	94.2	87.9	84.8	81.7	72.3	69.1	66	54.5	47.6	43	41.1	39.2	37.4	35.5	30.9	28.9	27.2	25.4	21.8	20
EC 329299	0.50%	-	++	+	+	++	++	++	+	+	+	++++	+	+++	++	+++	++	++	+++	++	+	+++	+++	++
	0.75%	-	++	+	+	++	++	++	-	+	+	+++	+	+++	+	++	+	+	++	+	+	++	+	++
	1%	+	++	+	+	++	++	++	+	+	+	+++	++	+++	++	++	+	++	+++	+	+	+++	++	+
	Control	+++	++	++	+	++	++	++	+	+	+	++++	+	+++	++	++	-	+	++	-	-	+	+	+
EC 318954	0.50%	+	++	-	+	++	++	++	+	+	+	++++	+++	+++	++	+++	+	++	+++	+	+	+++	++	++
	0.75%	+++	++	++	-	++	++	++	+	+	+	+++	+	+++	+	++	+	+	++	+	+	++	+	++
	1%	++	++	++	-	++	++	+	+	+	+	++++	+++	+++	++	+++	+	+	+++	+	+	+++	++	++
	Control	+++	++	++	-	++	++	+	+	-	+	+++	+	+	+	+	-	-	+	+	-	-	+	+
T 45-1	0.50%	+	++	+	-	+	++	+	+	+	+	+++	++	++	+	++	-	+	++	+	+	+++	+	+
	0.75%	+	++	++	-	++	+	+	+	+	++	++++	++	+++	++	+++	+	+	++	+	+	+++	++	+
	1%	+	++	++	+	++	++	+++	+	-	-	++++	+	+++	+++	+++	+	+	++++	+	+	+++	+++	++
	Control	+	++	++	+	+	+++	++	-	-	+	++++	+	+++	+++	+++	-	+	+++	-	+	+++	++	++
EC 407709	0.50%	+	++	++	+	+	+++	++	+	-	+	++++	+	+++	+++	+++	+	+	+++	+	+	+++	++	++
	0.75%	+	++	++	+	-	+++	++	+	-	+	+++	+	+++	++	+++	+	+	+++	+	+	+++	+++	++
	1%	-	++	++	+	+	++	++	+	++	+	++++	+	+++	++	+++	+	+	++	+	+	++	+	+
	Control	+	++	++	+	++	++	++	+	++	+	++++	+	+++	+++	+++	+	+	+++	+	++	+++	++	++
ISH 8020B	0.50%	+	++	++	+	++	++	++	+	++	+	+++	+	+++	++	+++	+	+	++	+	+	++	+	+
	0.75%	+	++	++	+	++	++	++	++	++	+	+++	+	+++	++	+++	+	+	++	+	+	+++	+	+
	1%	+	++	++	+	+	++	++	+	++	+	+++	+	+++	++	+++	+	+	+++	+	+	+++	++	+
	Control	+	++	++	+	+	++	++	+	++	+	++++	+	+++	++	+++	-	++	+++	+	+	+++	+	+

addition to control media (without NaCl salt) for callus induction. The results are presented in Tables 25-27.

F.1. Initial callusing response of various explants at different salinity level

F.1.1. Callus induction from petiole explants at 0.25% salinity

EC 318954 – Sixty percent explants developed into callus under control condition whereas at 0.25% salinity it increased to 90%. The callus was globular, pale green to pale yellow and attained an average size of 0.45 ± 0.25 cm as compared to the callus growing under control condition which was globular to fragile, pale yellow to green with an average size of 0.50 ± 0.43 cm.

EC 329299 - Fifty percent explants developed into callus under control condition and the callus was globular to fragile, pale green to yellow in colour with good growth and the average size of the callus was 0.40 ± 0.42 cm. Percent response for callus induction was same at 0.25% salinity also and the callus developed by 28th day was globular, pale green to pale yellow in colour with satisfactory growth and the average size of the callus was 0.25 ± 0.26 cm.

Wardan – Fifty percent explants developed into callus under control condition and the callus developed by 28th day were globular to fragile, pale green in colour with good growth rate and the average size of the callus was 0.40 ± 0.43 cm, whereas at 0.25% salinity, 40% of the explants developed into callus. The callus was mostly globular to fragile and pale green in colour. The growth rate was satisfactory with average size of 0.20 ± 0.29 cm.

F.1.2. Callus induction from hypocotyls explants at 0.25%

EC 318954 – Forty percent the explants developed into callus under control condition and the callus developed by 28th day was globular to fragile. The callus was pale yellow to green with good growth rate. The average size of the callus was 0.30 ± 0.40 cm. At 0.25% salinity, 80% of the explants developed into callus, the proliferated callus were globular to fragile. The callus was pale yellow to green. The growth of the callus was good and the average size of the callus was 0.72 ± 0.43 cm.

EC 329299 – Fifty percent explants developed into callus under control condition and the callus developed by 28th day was globular to fragile. The callus was pale yellow to green in colour. The growth rate was good and the average size of the callus was 0.42 ± 0.44 cm. At 0.25% salinity 40% of the explants developed into callus and the proliferated

callus was globular. The developed callus was pale yellow to green in colour. The growth of the callus was satisfactory and the average size of the calluses was 0.26 ± 0.36 cm.

Wardan – Fifty percent explants developed into globular to fragile callus under control condition. The callus was pale yellow to green in colour with good growth and the average size of the callus was 0.38 ± 0.41 cm whereas at 0.25% salinity 60% of the explants developed into pale yellow to green callus with marginal growth and the average size of 0.16 ± 0.14 cm.

F.1.3. Callus induction from petiole explants at 0.50% salinity

EC 318954 – Sixty percent explants developed into globular to fragile callus under control condition by 28th day. The callus had good growth rate and the average size of the callus was 0.50 ± 0.43 cm. At 0.50% salinity only 10% of the explants developed into globular and pale yellow callus. The growth of callus was marginal with the average size of 0.027 ± 0.08 cm.

EC 329299 – Fifty percent explants developed into globular to fragile callus under control condition by 28th days. The callus was mostly pale green to yellow. The growth of the callus was good. The average size of the callus was 0.40 ± 0.42 cm. At 0.50% salinity only 20% of the explants developed into mostly pale green callus. The growth of the callus was marginal and average size of the callus was 0.05 ± 11 cm.

Wardan – Fifty percent explants developed into globular to fragile callus under control condition. The callus was mostly pale green in colour. The growth rate of the callus was good. The average size of the callus was 0.40 ± 0.43 cm. At 0.50% salinity, 60% of the explants developed into globular to fragile and pale yellow to green callus. The growth rate of the callus was satisfactory with average size of 0.29 ± 0.31 cm.

F.1.4. Callus induction from hypocotyls explants at 0.50% salinity

EC 318954 – Forty percent explants developed into globular to fragile and yellow to green callus under control condition. The growth rate of the callus was good and the average size of the callus was 0.30 ± 0.40 cm. At 0.50% salinity, only 10% of the explants developed into callus which was compact and pale yellow in colour and marginal growth with average size of 0.02 ± 0.08 cm.

EC 329299 – Fifty percent of the explants developed into globular to fragile and pale yellow to green callus under control condition. The growth rate of the callus was good and the average size of the callus was 0.42 ± 0.44 cm whereas at 0.50 % salinity only

10% of the explants developed into mostly globular and pale green callus. The growth rate was marginal and the average size of the callus was 0.20 ± 0.07 cm.

Wardan – Sixty percent explants developed into globular to fragile and pale yellow to green callus under control condition. The growth rate of the callus was good and the average size was 0.38 ± 0.41 cm whereas at 0.50% salinity 30% of the explants developed into compact pale yellow to green callus. The average size of the callus was 0.15 cm ± 0.26.

F.1.5. Callus induction from petiole explants at 0.75% salinity

EC 318954 – Sixty percent of the explants cultured developed into globular to fragile and pale yellow to green callus under control condition. The growth rate of the callus was good and the average size of the callus was 0.50 ± 0.43 cm, whereas at 0.50% salinity only 30% of the explants developed into compact callus. The callus was mostly pale yellow to green. The growth rate was poor to marginal and the average size of the callus was 0.09 ± 0.15 cm.

EC 329299 – Fifty percent explants developed into globular to fragile callus under control condition. The callus was mostly pale green and yellow in colour with good growth rate and average size of 0.40 ± 0.42 cm, whereas at 0.75% salinity 60% of the explants developed into compact, pale yellow to green callus. The growth of the callus was slow to marginal and the average size of the callus was 0.34 ± 0.29 cm.

Wardan –Fifty percent explants developed into globular, fragile, pale green callus under control condition. The growth rate of the callus was good with average size of 0.40 ± 0.43 cm whereas at 0.75% salinity 70% of the explants responded and proliferated into globular, fragile callus which was pale yellow to green in colour mostly. The growth rate of the callus was satisfactory and the average size of the callus was 0.33 ± 0.26 cm.

F.1.6. Callus induction from hypocotyls explants at 0.75% salinity

EC 318954 – Forty percent explants developed into globular, fragile, pale yellow to green callus under control condition. The growth rate of the callus proliferation was good with average size of 0.30 ± 0.40 cm whereas at 0.75% salinity 30% of the explants developed into globular, pale yellow callus. The growth rate of the callus was marginal and the average size of the callus was 0.09 ± 0.14 cm.

EC 329299 – Fifty percent explants developed into globular, fragile, pale yellow to green callus, under control condition. The growth rate of the callus proliferation was good and the average size of the callus was 0.42 ± 0.44 cm whereas at 0.75% salinity only 20% of

the explants developed into globular to fragile callus. The callus was pale yellow to green in colour. The growth rate of callus proliferation was marginal and the size of the callus was 0.11 ± 0.24 cm.

Wardan Fifty percent explants developed into globular, fragile, pale yellow to green callus. The growth of callus was good and the average size of the callus was 0.38 ± 0.41 cm whereas at 0.75% salinity 40% of the explants proliferated into globular, fragile, pale yellow to green callus. The growth rate of callus was good and the average size of the callus was 0.29 ± 0.46 cm.

F.2. Response of callus, developed in saline condition, to higher salinity

EC 318954

Petiole derived callus developed under 0.25% salinity were transferred to 0.50% salinity. Of the total calli cultured, 83.3% responded positively and proliferated into globular, compact, pale yellow to green callus. The growth rate ranged from good to marginal and the average size of the callus was 0.43 cm ± 0.33. However, such calli when transferred to regenerating LSP_3 showed slower response and 62.5% of the calli proliferated into compact, pale yellow to pale green callus. The growth rate was slow and marginal. The average size of the callus was 0.15 cm ± 0.13.

Hypocotyls derived calli developed at 0.25% salinity was transferred to 0.50% salinity. Of the total calli cultured, 83.3% responded positively to higher level of salinity and proliferated into globular, fragile callus. The callus was pale green to yellow in colour. The growth rate was good and calli grown to 0.61 ±0.33 cm sizes. Such hypocotyls derived callus when transferred to LSP_3 showed 83.3% response. The callus was globular and fragile bearing a few which were compact. The callus was pale yellow to pale green in colour. The growth rate of the callus was satisfactory and it grew to 0.40 ±0.25 cm sizes.

The petiole-derived calli developed at 0.50% salinity, when transferred to 0.75% salinity responded positively. The proliferated callus was globular and fragile, pale yellow to green in colour and the growth rate was good to satisfactory. The average size of the callus was 0.64 ± 0.15 cm. Such hypocotyls derived calli responded well to LSP_3 medium also and 66.6% the calli responded positively. The callus was mostly globular and fragile, pale yellow to green and the growth rate of the callus was good to satisfactory. The average size of the callus was 0.41 ± 0.33 cm.

The petiole-derived calli developed at 0.75% salinity were transferred to LSP_3 medium and 37.5% of the callus responded positively to regenerating media. The callus was mostly globular and compact, pale yellow to pale green in colour. The growth rate was slow and ranged from satisfactory to marginal. The average size of the callus was 0.12 cm ± 0.19.

The hypocotyls derived calli developed at 0.25% salinity were transferred to 0.50% salinity wherein 75.0% of the calli responded positively to higher levels of salinity. The proliferating callus was globular, fragile, pale yellow to green in colour. The growth rate was good to marginal and the average size of the callus was 0.51 ± 0.35 cm. Such hypocotyls derived calli developed at 0.25% salinity when transferred to LSP_3, showed average response and 44.4% of the callus transferred responded positively to regenerating media. The proliferating callus was globular, fragile and pale yellow in colour. The growth rate was satisfactory to marginal and the average size of the callus was 0.18 to 0.27 cm.

The hypocotyls derived calli developed at 0.75% salinity were transferred to LSP_3. 75.0% of the callus transferred responded positively to regenerating media. The proliferating callus was globular fragile mostly and some part compact in nature. The callus was pale yellow to green in colour. The growth rate of the callus was very good and the average size of the callus was 0.63 cm ± 0.48.

Wardan

Petiole derived calli developed at 0.25% salinity was cultured at 0.50% salinity wherein, 77.7% of the callus responded positively. The callus was globular and compact. The callus was pale yellow to green in colour. The average size of the callus was 0.57 ± 0.38 cm and the growth rate of the callus proliferation was good to satisfactory. Such petiole-derived calli developed at 0.25% salinity were also cultured on LSP3 medium wherein 57.1% of the callus responded positively. The callus was globular and compact. The callus was pale green in colour. The growth of callus was slow, marginal and with average size of 0.23 ± 0.26 cm.

Petiole derived calli developed at 0.50% salinity was cultured at 0.75% salinity wherein 52.8% of the callus responded positively. The proliferating callus was mostly globular and fragile. The callus was pale yellow to green in colour, the average size of the callus was 0.36 ± 0.37 cm and the growth rate of the callus was very good.

Petiole derived calli developed at 0.50% salinity was cultured on LSP_3 also. In this medium only 23.8% of the callus responded and developed in pale yellow to green calli. The growth of callus proliferation was marginal and the average size of the callus was 0.16 ± 0.30 cm. Petiole derived calli developed at 0.75% L_2 were sub cultured on LSP_3 wherein only 11% of the callus responded to regenerating media. The callus was green in colour. The growth rate of callus proliferation was slow and satisfactory. The average size of callus was 0.06 cm ± 0.19.

Hypocotyls derived calli developed at 0.25% salinity when cultured on 0.50% salinity, only 25.0% of the callus responded and the callus was pale green in colour. The growth rate was slow and marginal. The average size of the callus was 0.065 ± 0.13 cm. Such hypocotyls derived calli developed at 0.25% salinity when cultured on LSP_3, 33% of the callus responded and the callus was globular and fragile and pale yellow in colour. The growth rate of callus proliferation was slow, marginal and the average size of the callus was 0.07 ± 0.12 cm.

Hypocotyls derived calli developed at 0.50% salinity were cultured on 0.75% salinity wherein 33.3% of the callus responded to increased salinity level and the callus was globular and compact. The callus was pale yellow and pale green mostly. The growth rate of callus was slow and satisfactory. The average size of callus was 0.20 ± 0.35 cm. Such hypocotyls derived calli developed at 0.50% salinity were also cultured on LSP_3 wherein 66.6% of the callus responded to regenerating media and pale yellow and green callus was obtained. The growth rate of callus proliferation was good and the average size of the callus was 0.48 ± 0.42 cm. Response of hypocotyls derived calli developed at 0.75% salinity on LSP_3 medium was still poor and only 28.6% of the callus responded to regenerating media. The proliferating callus was globular and compact. The callus was pale green in colour mostly. The growth rate of callus was good; the average size of the callus was 0.14 ± 0.29 cm.

F.3. *In vitro* response of callus to further increased salinity

EC 318954 (Petiole)

0.25% L_2, 0.50% L_2 to 0.75% L_2 – 53.8% of the callus responded positively to increased salinity treatment. The callus was globular, fragile and pale green in colour. The growth of the callus was good to marginal, the average size of the callus was 0.25 ± 0.30 cm.

0.25% L_2, LSP_3 to LSe – 66.6% of the callus responded to SEIM media and the proliferating callus was fragile and compact. The callus was mostly pale green in colour. The growth rate of callus growth was just satisfactory and the average size of the callus was 0.25 ± 0.21 cm.

0.25% L_2, 0.50% L_2 to LSP_3 - All the calli responded to regenerating media, the proliferating calli was mostly fragile though some portion was compact. The callus was mostly pale yellow. The growth rate was slow, marginal and the average size of the callus was 0.09 ± 0.12 cm.

0.25% L_2, 0.50% L_2 to LSe - 50% of the total callus responded to SEIM media and the proliferating callus was mostly globular and somewhat compact in nature. The callus was pale yellow to green in colour. The growth rate was slow and just satisfactory; the average size of the callus was 0.53 ± 0.22 cm.

Hypocotyls explants

0.25% L_2, 0.50% L_2 to 0.75% L_2 – 63.3% of the total callus transferred responded positively to higher salinity treatments and the proliferating callus was mostly globular and some were compact in nature. The callus was mostly pale green in colour. The growth rate of callus proliferation was good to marginal and the average size was 0.20 ± 0.23 cm.

0.25% L_2, 0.50 % L_2 to LSP_3 – All the callus transferred responded positively to regenerating media and the proliferating callus was globular mostly leaving some which were compact in nature. The callus was pale yellow to green. The growth rate was good mostly and the average size of the callus was 0.57 ± 0.17 cm.

0.25% L_2, 0.50% L_2 to LSe - All the callus responded positively to SEIM media and the callus obtained was globular, mostly fragile except a few compact calluses. The callus was pale green and pale yellow in colour. The growth rate of callus proliferation was good mostly and the average size of callus was 042 ± 0.17 cm.

0.25% L_2, LSP_3 to LSe – All the callus responded to SEIM media and the proliferated callus was mostly compact. The callus was pale yellow to pale green in colour. The growth rate of callus proliferation was slow and just satisfactory; the size of callus was 0.41 ± 0.15 cm.

0.25% L_2, LSP_3 to LSP_3 – All the callus transferred again into regenerating media responded positively and mostly compact type of callus was obtained. The callus was pale

green to pale yellow in colour. The growth rate of callus proliferation was good to satisfactory and the average size of callus was 0.46 ± 0.38 cm.

0.75% L_2, LSP_3 to LSP_3 – 40% of the total callus responded to regenerating media and the proliferating callus was mostly compact. The growth rate of callus proliferation was slow to marginal. The average size of callus was 0.09 ± 0.13 cm.

EC 329299 (Petiole)

0.25% L_2, 0.50% L_2 to 0.75% L_2 – 84.6% of the callus responded positively at higher salinity in callus induction media and the callus was mostly compact though few calli were globular in nature. The callus was mostly pale green to green in colour. The growth rate was good to satisfactory and the average size of the callus was 0.48 ± 0.29 cm.

0.25% L_2, 0.50% L_2 to LSP_3 – All the callus responded positively to regenerating media and the proliferated callus was mostly green and few of the calluses were pale green in colour. The growth rate was good to satisfactory and the average size of the callus were 0.65 ± 0.16 cm.

0.25% L_2, 0.50% L_2 to LSe – 72.7% of the total callus responded to SEIM media and the proliferated callus was compact and hyaline. The growth rate of callus proliferation was good to marginal and the average size of callus was 0.36 ± 0.29 cm.

0.25% L_2, LSP_3 to LSe – All the callus responded to SEIM media and proliferation of callus was observed and mostly compact callus was observed. The callus was pale green to green in colour. The growth rate was slow and satisfactory; the average size of the callus was 0.48 ± 0.24 cm.

Hypocotyls

0.25% L2,0. 50% L2 to 0.75% L2 – 50% of the callus responded positively to increased salinity level in shoot induction media and the callus was mostly compact except a few fragile calli. The callus was pale green to pale yellow in colour. The growth rate of callus proliferation was very good and the average size of callus was 0.34 ± 0.38 cm.

0.25%L_2, 0.50% L_2 to LSP_3 - All the callus responded positively to regenerating media. The callus was mostly globular and fragile. The callus was pale green to pale yellow in colour. The growth rate was good to marginal and the average size was 0.45 ± 0.24 cm.

0.25% L_2, 0.50% L_2 to LSe – 50% of the callus responded positively to SEIM media, the proliferating callus was compact and globular. The callus was pale yellow to pale green in colour mostly. The growth rate was slow and satisfactory, the average size of callus was 0.13 ± 0.15 cm.

0.75% L_2, LSP_3 to LSe – 62.5% of the callus responded to SEIM media and the callus was compact and globular mostly. The callus was pale green to pale yellow in colour mostly. The growth rate of callus good to marginal and the callus size was 0.21 ± 0.28 cm.

0.75% L_2, LSP_3 to LSP_3 - 33.3 % of the callus responded to repeated regenerating media. The callus was compact in nature; pale yellow to green in colour but the growth rate of callus was poor and slow. The average size of the callus was 0.005 cm ± 0.09.

Wardan (Petiole)

0.25% L_2, 0.50% L_2 to 0.75% L_2 - All the callus responded positively to increased salinity treatments in callus induction media. The proliferating callus was globular to fragile, pale green to pale yellow in colour. The growth rate of callus was slow and satisfactory. The average size of the callus was 0.32 ± 0.11 cm.

0.25% L_2, 0.50% L_2 to LSP3 - All the callus responded to regenerating media and compact to globular callus was obtained by 28th day. The callus was green to pale green in colour the growth rate of callus was good to satisfactory. The average size of callus was 0.66 ± 0.18 cm.

0.25% L_2, 0.50% L_2 to LSe - 80% the callus responded positively to SEIM media and the callus was mostly compact to globular. The callus was pale green in colour. The growth rate was slow and just satisfactory; the average size of the callus was 0.21 ± 0.16 cm.

0.50% L_2, 0.75% L_2 to LSP_3 – 41.6 % of the callus to regenerating media responded and the callus was globular to fragile. The callus was pale green to pale yellow in colour. The growth rate of callus was slow and satisfactory; the average size of the callus was 0.10 ± 0.14 cm.

0.50% L_2, 0.75% L_2 to LSe - Only 33.3% of the callus responded to SEIM media though the growth of callus was very slow. The callus was globular to fragile in nature and pale green in colour. The growth rate was marginal and the average size of the callus was 0.07 ± 0.10 cm.

0.50% L_2, LSP_3 to LSP_3 - the entire callus responded to regenerating media but the growth of callus was slow. The callus was globular and fragile, mostly pale green in colour with very marginal growth. The average size of the callus was 0.20 ± 0.03 cm.

0.50% L_2, LSP_3 to LSe - 50% of the callus responded to SEIM media. The callus obtained by 28th day was globular and fragile, pale yellow to green in colour. The growth

Table 25. *In vitro* callusing response in Egyptian clover genotypes.

Genotype	Media	Treatment	Explant Source	No. of explant	Callus Response in % 1 -7 Days	7 -14 Days	14 -21 Days	21 -28 Days	Callus Characteristics Nature	Colour	Growth	Callus Size
EC 318954	L2	0.25%	Petiole	10	80	100	90	90	Globular	PG, PY	+++	0.45 ± 0.25
EC 329299	L2	0.25%	Petiole	10	90	80	70	50	Globular	PG, PY	++	0.25 ± 0.26
Wardan	L2	0.25%	Petiole	10	90	90	70	40	Globular, Fragile	PG	++	0.20 ± 0.29
EC 318954	L2	0.25%	Hypocotyl	10	100	100	80	80	Globular, Fragile	PY, G	+++	0.72 ± 0.43
EC 329299	L2	0.25%	Hypocotyl	10	100	80	60	40	Globular	PY, G	++	0.26 ± 0.36
Wardan	L2	0.25%	Hypocotyl	10	100	80	70	60	Globular	PY, G	+	0.16 ± 0.14
EC 318954	L2	0.50%	Petiole	10	30	20	10	10	Globular	PY	+	0.027 ± 0.08
EC 329299	L2	0.50%	Petiole	10	100	90	50	20	Globular	PG	+	0.05 ± 0.11
Wardan	L2	0.50%	Petiole	10	100	60	60	60	Globular, Fragile	PY, G	++	0.29 ± 0.31
EC 318954	L2	0.50%	Hypocotyl	10	80	50	30	10	Compact	PY	+	0.02 ± 0.08
EC 329299	L2	0.50%	Hypocotyl	10	100	100	10	10	Globular	PG	+	0.20 ± 0.07
Wardan	L2	0.50%	Hypocotyl	10	90	60	40	30	Compact	PY, G	++	0.15 ± 0.26
EC 318954	L2	0.75%	Petiole	10	40	50	40	30	Compact	PY,G	+	0.09 ± 0.15
EC 329299	L2	0.75%	Petiole	10	80	80	60	60	Compact	PY,G	+	0.34 ± 0.29
Wardan	L2	0.75%	Petiole	10	100	90	70	70	Globular	PY,G	++	0.33 ± 0.26
EC 318954	L2	0.75%	Hypocotyl	10	60	20	40	30	Globular	PY	+	0.09 ± 0.14
EC 329299	L2	0.75%	Hypocotyl	10	60	20	20	20	Globular,Fragile	PY,G	+	0.11 ± 0.24
Wardan	L2	0.75%	Hypocotyl	10	50	50	40	40	Globular,Fragile	PY,G	++++	0.29 ± 0.46
EC 318954	L2	Control	Petiole	10	90	70	60	60	Globular,Fragile	PY,G	+++	0.50 ± 0.43
EC 329299	L2	Control	Petiole	10	100	50	50	50	Globular, Fragile	PG,Y	+++	0.40 ± 0.42
Wardan	L2	Control	Petiole	10	100	50	50	50	Globular, Fragile	PG	+++	0.40 ± 0.43
EC 318954	L2	Control	Hypocotyl	10	60	60	40	40	Globular,Fragile	PY,G	+++	0.30 ± 0.40
EC 329299	L2	Control	Hypocoytl	10	100	60	50	50	Globular,Fragile	PY,G	+++	0.42 ± 0.44
Wardan	L2	Control	Hypocotyl	10	100	50	60	50	Globular,Fragile	PY,G	+++	0.38 ± 0.41

PG= pale green, PY = pale yellow, G= green, ++++ = phenomenol growth, +++ = good growth, ++ = satisfactory, + = poor growth

Table 26. Response of callus, induced in saline condition, to higher salinity

Prior Treatment	Present Treatments	Explant Source	No. of tubescyultured	Response in % 1 -7 Days	7 - 14 Days	14 - 21 Days	21 - 28 Days	Callus Characteristics Nature	Colour	Growth	Callus size
EC 318954											
0.25% L2	0.50% L	Petiole	12	83.3	83.3	83.3	83.3	Globular, Compact	PY,G	3+ to 1+	0.43 ± 0.33
0.25% L2	LSP3	Petiole	8	75.0	62.5	62.5	62.5	Compact	PY, PG	1+	0.15 ± 0.13
0.25% L2	0.50% L2	Hypocotyl	18	100.0	88.0	83.3	83.3	Globular, Fragile,Compact	PG,PY	4+ to 2+	0.61 ± 0.33
0.25% L2	LSP3	Hypocotyl	14	85.7	85.7	85.7	71.4	Globular, Fragile,Compact	PG, Y	3+ to 1+	0.30 ± 0.26
0.75% L2	LSP3	Hypocotyl	6	83.3	83.3	83.3	83.3	Globular,Fragile	PY.	3+ 2+	0.64 ±0.32
EC 329299											
0.25% L2	0.50% L2	Petiole	8	75.0	87.5	87.5	87.5	Globular, Fragile, Compact	PY,G	3+ to 1+	0.58 ± 0.30
0.25% L2	LSP3	Petiole	6	83.3	83.3	83.3	83.3	Globular, Fragile, Compact	PY,PG	2+ to 1+	0.40 ± 0.25
0.25%L2	0.50% L2	Hypocotyl	12	91.6	91.6	91.6	75.0	Globular, Fragile	PY, G	3+ to 1+	0.51 ± 0.35
0.25%L2	LSP3	Hypocotyl	9	66.6	44.4	44.4	44.4	Globular, Fragile	PY	2+ to 1+	0.18 ± 0.27
0.50% L2	0.75% L2	Petiole	6	100.0	100.0	100.0	100.0	Globular, Fragile	PY, G	3+ to 2+	0.64 ± 0.15
0.50%L2	LSP3	Petiole	6	100.0	100.0	100.0	66.6	Globular, Fragile	PY, G	3+ to2+	0.41 ± 0.33
0.75% L2	LSP3	Petiole	8	87.5	62.5	37.5	37.5	Globular,Compact,Fragile	PY, PG	2+ to 1+	0.12 ± 0.19
0.75% L2	LSP3	Hypocotyl	4	75.0	75.0	75.0	75.0	Globular, Fragile, Compact	PY, G	4+ to 3+	0.63 ± 0.48
Wardan											
0.25% L2	0.50% L2	Petiole	9	100.0	88.8	88.8	77.7	Globular, Compact	PY, G	3+ to 2+	0.57 ± 0.38
0.25% L2	LSP3	Petiole	7	85.7	71.4	57.1	57.1	Globular, Compact	PG	1+ to 2+	0.23 ± 0.26
0.25% L2	0.50% L2	Hypocotyl	4	50.0	25.0	25.0	25.0	Globular, Fragile	PG	1+	0.065 ± 0.13
0.25% L2	LSP3	Hypocotyl	3	33.3	33.3	33.3	33.3	Globular, Fragile	PY	1+	0.07 ± 0.12
0.50% L2	0.75% L2	Petiole	17	82.4	76.5	76.5	52.8	Globular, Fragile	PY, G	4+ to 2+	0.36 ± 0.37
0.50% L2	LSP3	Petiole	21	66.6	57.1	38.1	23.8	Globular, Fragile	PY, G	3+ to 1+	0.16 ± 0.30
0.50% L2	0.75% L2	Hypocotyl	3	66.6	66.6	33.3	33.3	Globular, Compact	PY,PG	2+	0.20 ± 0.35
0.50% L2	LSP3	Hypocotyl	3	66.6	66.6	66.6	66.6	Globular, Compact	PY, G	3+ to 2+	0.48 ± 0.42
0.75% L2	LSP3	Petiole	9	55.5	22.2	11.0	11.0	Compact	G	2+	0.06 ± 0.19
0.75% L2	LSP3	Hypocotyl	7	11.1	28.6	28.6	28.6	Globular, Compact	PG	3+ to 2+	0.14 ± 0.29

PY=pale yellow, G= green, 3+ = good growth, 2+ = satisfactory, 1+ =poor growth

Table 27. Response of callus, induced in saline condition, to further higher salinity

Prior Treatment	Present Treatment	Explant Source	No. of callus	Response in % 1 - 7 Days	7 - 14 Days	14 - 21 Days	21 - 28 Days	Callus Characteristic Nature	Colour	Growth	Callus size
EC 318954											
0.25%L2,0.50%L2	0.75%L2	Petiole	13	69.2	61.5	53.8	53.8	Globular,Fragile	PG	3+ to 1+	0.25 ± 0.30
0.25%L2, LSP3	Lse	Petiole	3	100.0	100.0	100.0	66.6	Fragile,Compact	PG	2+ 1+	0.21 ± 0.21
0.25%L2, 0.50%L2	LSP3	Petiole	5	100.0	100.0	100.0	100.0	Fragile,Compact	PY	1+	0.09 ± 0.12
0.25%L2,0.50%L2	LSe	Petiole	6	66.6	66.6	66.6	50.0	Globular,Compact	PY,G	2+ 1+	0.53 ± 0.22
0.25%L2, 0.50%L2	0.75% L2	Hypocotyl	11	90.9	81.8	72.7	63.6	Globular,Compact	PG	3+ to 1+	0.20 ± 0.23
0.25%L2, 0.50%L2	LSP3	Hypocotyl	5	100.0	100.0	100.0	100.0	Globular,Compact	PY,G	3+ 1+	0.57 ± 0.17
0.25%L2,0.50%L2	LSe	Hypocotyl	6	100.0	100.0	100.0	100.0	Globular,Compact,Fragile	PG,PY	3+ to1+	0.42 ± 0.17
0.25%L2,LSP3	LSe	Hypocotyl	3	100.0	100.0	100.0	100.0	Compact	PY,PG	2+ to1+	0.41 ± 0.15
0.25%L2,LSP3	LSP3	Hypocotyl	6	100.0	100.0	66.6	100.0	Compact,Globular	PG,PY	3+ to2+	0.46 ± 0.38
0.75%L2,LSP3	LSP3	Hypocotyl	5	100.0	100.0	80.0	40.0	Compact	PG	1+	0.09 ± 0.13
0.75%L2,LSP3	LSe	Hypocotyl	3	66.6	33.3	0.0	0.0	0	0	0	0
EC 329299											
0.25%L2,0.50%L2	0.75%L2	Petiole	13	84.6	84.6	84.6	84.6	Compact,Globular	PG,G	3+ to 2+	0.48 ± 0.29
0.25%L2,0.50%L2	LSP3	Petiole	6	100.0	100.0	100.0	100.0	Compact,Globular	G,PG	3+ to 2+	0.65 ± 0.16
0.25%L2,0.50%L2	Lse	Petiole	11	100.0	81.8	72.7	72.7	Compact,Globular,Hyaline	PG,G	3+ to 1+	0.36 ± 0.29
0.25%L2,LSP3	LSe	Petiole	3	100.0	100.0	100.0	100.0	Compact,Globular	PG,G	2+ to 1+	0.48 ± 0.24
0.25%L2,LSP3	LSP3	Petiole	3	100.0	100.0	100.0	100.0	Compact,Globular,Hyaline	PG	3+ to 1+	0.69 ± 0.08
0.25%L2,0.50%L2	0.75%L2	Hypocotyl	16	81.3	75.0	50.0	50.0	Compact,Globular,Fragile	PG,PY	4+ to 2+	0.34 ± 0.38
0.25%L2,0.50%L2	LSP3	Hypocotyl	5	100.0	100.0	100.0	100.0	Globular,Fragile,Hyaline	PG,PY	3+ to 1+	0.45 ± 0.24
0.25%L2,0.50%L2	LSe	Hypocotyl	10	80.0	60.0	50.0	50.0	Compact,Globular	PY,PG	2+ to 1+	0.13 ± 0.15
0.75%L2,LSP3	LSe	Hypocotyl	8	100.0	75.0	75.0	62.5	Compact,Globular,Fragile	PG,PY	3+ to 1+	0.21 ± 0.28
0.75%L2,LSP3	LSP3	Hypocotyl	3	100.0	66.6	33.3	33.3	Compact,	PY, g	1+	0.05 ± 0.09
Wardan											
0.25%L2,0.50%L2	0.75%L2	Petiole	4	100.0	100.0	100.0	100.0	Fragile,Globular	PG,PY	2+ to 1+	0.32 ± 0.11
0.25%L2,0.50%L2	LSP3	Petiole	3	100.0	100.0	100.0	100.0	Compact,Globular	G,PG	3+ to 2+	0.66 ± 0.18
0.25%L2,0.50%L2	Lse	Petiole	5	100.0	100.0	100.0	80.0	Compact,Globular,Fragile	PG	2+ to 1+	0.21 ± 0.16
0.50%L2,0.75L2	LSP3	Petiole	12	58.3	50.0	41.6	41.6	Globular,Fragile	PG,PY	2+ to 1+	0.10 ± 0.14
0.50%L2,0.75L2	LSe	Petiole	9	33.3	33.3	33.3	33.3	Globular,Fragile	PG	1+	0.07 ± 0.10
0.50%,LSP3	LSP3	Petiole	3	100.0	100.0	100.0	100.0	Globular,Fragile	PG	1+	0.20 ± 0.03
0.50%L2,LSP3	LSe	Petiole	2	50.0	50.0	50.0	50.0	Globular,Fragile	PY,G	1+	0.11 ± 0.16
0.50%L2,0.75L2	LSP3	Hypocotyl	4	25.0	25.0	25.0	25.0	Fragile,Globular	PG	2+	0.14 ± 0.29

PY=pale yellow, G= green, 4+ = phenomenol growth, 3+ = good growth, 2+ = satisfactory, 1+ =poor growth

rate of callus proliferation was very slow and marginal. The average size of the callus was 0.11 ± 0.16 cm.

0.50% L_2,0.75 L_2 to LSP_3 - 25% of the callus transferred responded to regenerating media. The callus obtained by 28th day was fragile and globular, pale – green in colour. The growth rate of callus proliferation was just satisfactory and the average size of the callus was 0.14 ± 0.29 cm.

G. *In vitro* embryo culture response under saline *vis-à-vis* normal condition.

Flowers of the three ecotypes of *Trifolium* i.e., EC 329299 (Saidi), EC 318954 (Fahli) and Wardan (Mescavi) were brought to laboratory. The ovules with embryo at cotyledonary stage were excised, surface sterilized under aseptic conditions and inoculated on MS basal media supplemented with 0.3% Kinetin. The medium was divided into 4 equal parts, 3 parts were supplemented with 0.25%, 0.50% and 0.75% NaCl whereas the 4th part was taken without salt supplementation as control. Two ovules were cultured per tube and 20 to 60 ovules were cultured per genotype for every treatment. The results of the study are presented in Table 28-30.

G.1. Initial embryo culture response

EC 318954

Germination: 69.5% of ovules cultured under control condition germinated by 7th day of inoculation, whereas at 0.25%, 0.50% and 0.75% salinity treatments, 58.6, 39.3 and 12% of the ovules germinated respectively.

Plumule length: The length of plumule under control condition ranged from 1.3 to 5 cm, with an average length of 2.8 cm $\pm$ 1.06. The plumule length of plants growing at 0.25%, ranged from 1.2 to 3.2 cm and the average length was 2.11 cm $\pm$ 0.59. At 0.50% salinity the plumule length ranged from 1.0 to 2.1 cm and the average length was 1.5 cm $\pm$ 0.42 whereas at 0.75% salinity no plant survived till the 20th day.

Radicle length: The average length of the radicle in the plants growing under control condition was 1.46 cm $\pm$ 0.84 and it ranged from 0.5 to 3.1 cm. Among the plants growing at 0.25%, 0.50% salinity the average length of roots was 1.51 $\pm$ 0.82 and 1.1 cm $\pm$ 1.04 which ranged from 0.5 to 2.8 and 0.4 to 2.3 cm respectively. At 0.75% salinity no plant survived till 20th day.

Number of leaves: The average number of leaves in the plant growing under control condition was 2.8 $\pm$ 1.33 and the range was 1 to 4 leaves whereas at 0.25% and 0.50 %

salinity the average was 2.1 ± 0.96 and 1.37 ± 0.51 and the range was 1 to 4 and 1-2 leaves respectively.

Mortality: Under control condition 13.8% mortality of embryos was observed whereas at 0.25%, 0.50% and 0.75% salinity, 32.0, 57.1 and 100% mortality was observed.

EC 329299

Germination: 77.7% of the ovules cultured germinated under control condition which was progressively reduced to 58.3, 42.5 and 12.5% at 0.25%, 0.50% and 0.75% salinity respectively.

Plumule length: The average length of the plumule under control condition was 2.55 cm ± 1.27 and the range of plumule length ranged from 0.7 cm to 5.4 cm. At 0.25%, 0.50% and 0.75% salinity the average plumule length of the plants was 1.87 cm ± 0.81, 0.8 cm ± 0.26 and 2.6 cm ± 0 whereas the plumule length ranged from 0.9 to 31 cm, 0.6 to 1.1 cm and 0 to 2.6 cm respectively.

Radicle length: The average length of the radicle in the plants growing under control condition was 2.37 cm ± 1.62 and it ranged from 0.3 to 4.8 cm. At 0.25% and 0.50% salinity the average radicle length was 1.42 cm ± 0.98 and 1.45 cm ± 1.62 whereas the length of radicle in the plants ranged from 0.3 to 2.8 cm and 0.3 to 2.6 cm respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 2.12 cm ± 0.95 and the range of leaves present in the plants varied from 1 to 4. At 0.25%, 0.50% and 0.75% salinity the average number of leaves were 2 ± 1.26, 1 ± 0 and 3 ± 0 whereas the number of leaves in the plants varied from 1 to 4, 0-1 and 0-3 respectively.

Mortality: By 20th day 18.2 % mortality was observed under control condition whereas at 0.25%, 0.50% and 0.75% salinity conditions 42.9, 70.5 and 80.0 mortality was observed.

Wardan

Germination: 85.7% of the ovules germinated by 7th day of inoculation under control condition whereas at 0.25%, 0.50% and 0.75% salinity 83.3, 73.3 and 27.3% of the ovules germinated.

Plumule length: The average plumule length of plants growing under control condition was 2.79 ± 1.07 and the plumule length ranged from 1.3 to 4.9 cm. At 0.25%, 0.50% and 0.75% salinity the average plumule length of the plants was 2.05cm ± 0.6, 1.08 cm ± 0.33

and 1.2 cm whereas the plumule length in the plants varied from 1 to 3.1, 0.9 to 1.6 and 0 to 1.2 respectively.

Radicle length: The average length of radicle in the plants growing under control condition was 2.55 cm ± 1.32 and it ranged from 0.8 to 5.5 cm. At 0.25%, 0.50% and 0.75% salinity the average radicle length of the plants was 2.44± 0.96, 2.3 cm ± 0 and 1.1 cm ± 0.14 whereas the length of radicle in the plants ranged from 0.8 to 3.9 cm, 0 to 2.3 cm and 1 to 1.2 cm respectively.

Number of leaves: The average number of leaves in the plants growing under control condition was 2.75 ± 1.11 and the range of leaves in the plants were 1 to 5. At 0.25%, 0.50% and 0.75% salinity the average number of leaves were 2.7 ± 1.68, 1 ± 0 and 0 whereas the number of leaves in the plants ranged from 1 to 7, 0 to 1 and 0 respectively.

Mortality: 16.7% to the plants degenerated under control condition whereas at 0.25%, 0.50% and 0.75% salinity 32, 59.1 and 66.7% of the plants degenerated.

G.2. Sub culturing response

The surviving plants were transformed to MS media and RL media supplemented with required amount of NaCl for 0.25%, 0.50% and 0.75% salinity treatments. The plants were transferred to the same level of treatment in which they were growing. The results of the study are presented in the table –

EC 318954

0.25% - Out of four sub cultured plants, one plant died by 20th day. The average plumule length of the surviving plants was 3.36 cm, radicle length was 3.66 cm and the number of leaves was 5.66.

0.50% - Only 1 plants could be transferred which degenerated by 20th day.

Control - 10 plants were transferred and no mortality was observed till 20th day. The average plumule length of the plants was 6.55 cm, the average radicle length was 4.1 cm whereas the average number of leaves in the plants was 7.3.

EC 329299

0.25% - Out of four plants subcultures three degenerated by 20th day. The surviving plant possessed 3.3 cm long plumule, 4.1 cm long radicle and three leaves.

0.50% - Two plants were sub cultured to MS media, which degenerated by 20th day.

Control – Thirteen plants were sub cultured of which 30.76% plants perished by 20th day. The average plumule length of the plants was 4.4 cm, the radicle length was 4.23 cm and the average number of leaves in the plants was 4.88.

Wardan

0.25% - Twelve plants were sub cultured, of which 8.33% degenerated by 20^{th} day. The average length of plumule of the plants was 3.23 cm, radicle length was 3.39 cm and the average number of leaves was 4.2.

0.50% - One plant could be sub cultured which survived till 20th day. The plumule length of the plant was 2.4 cm, radicle length was 2.9 cm and the number of leaves was 2.

0.75% - Two plants were sub cultured and by 20^{th} day both the plants degenerated.

Control – Twenty plants were sub cultured of which 15% degenerated. The average plumule length of the plants was 5.11 cm, radicle length was 4.5 cm and the number of leaves was 6.42.

G.3. Response to rooting media

The surviving plants from the 1^{st} experiment, which failed to develop roots, were transferred into RL media supplemented with required amount of NaCl for 0.25% and 0.50% salinity. The control plants, which had poor growth of roots, were also transferred in RL media without NaCl supplementation as control. The data was recorded on 20^{th} day after transfer.

EC 318954

0.25% - Ten plants were sub cultured on RL media of which 30% showed mortality by 20^{th} day. The average plumule length of the plants was 5.76 cm, radicle length was 2.26 cm and the average number of leaves was 5.66.

0.50% - Five plants were sub cultured, of which 80% degenerated by 20^{th} day. The average plumule length of the plants was 2.7 cm, the radicle length was 0.6 cm and the average number of leaves was 5.

Control – 10 plants were sub cultured and no mortality was observed. The average plumule length of the plants was 6.55 cm, the radicle length was 4.1 and the average number of leaves in the plants was 7.3.

EC 329299

0.25% - One plant was transferred and by 20^{th} day the plant degenerated.

0.50% - One plant was transferred and by 20^{th} day the plant degenerated.

Control – 13 plants were sub cultured of which 30.76% degenerated by 20^{th} day. The average plumule length of the plants was 4.4 cm, the radicle length was 4.23 cm and the average number of leaves in the plants was 4.89.

Table 28. *In vitro* embryo culture response under saline conditions in Egyptian clover genotypes.

			Germination on different days							Plumule length			Radicle length			No. of leaves		
Genotype	Treatment	No of ovules cultured	3	4	5	6	7	% germination	% Mortality on 20th day	Range	Mean	S D	Range	Mean	S D	Range	Mean	S D
EC 318954	0.25%	58	2	17	30	34	34	58.6	32.0	1.2 - 3.2	2.11	0.59	0.5 - 2.8	1.51	0.82	1-- 4	2.1	0.96
	0.50%	56	-	-	3	17	22	39.3	57.1	1 - 2.1	1.5	0.42	0.4 - 2.3	1.1	1.04	1 -- 2	1.37	0.51
	0.75%	50	-	-	-	3	3	12.0	100.0									
	Control	44	10	18	29	29	29	65.9	13.8	1.3 - 5	2.8	1.06	0.5 - 3.1	1.46	0.84	1 --4	2.8	1.33
			-	-	-	-	-	-	-									
EC 329299	0.25%	48	-	12	27	28	28	58.3	42.9	0.9 - 3.1	1.87	0.81	0.3 -- 2.8	1.42	0.98	1 -- 4	2	1.26
	0.50%	40	-	1	12	17	17	42.5	70.5	0.6 - 1.1	0.8	0.26	0.3 - 2.6	1.45	1.62	0 - 1	1	0
	0.75%	40	-	-	-	3	5	12.5	80.0		2.6	0					3	0
	Control	44	15	32	34	34	34	77.3	18.2	0.7 - 5.4	2.55	1.27	0.3 - 4.8	2.37	1.62	1 --4	2.12	0.95
			-	-	-	-	-	-	-									
Wardan	0.25%	30	1	15	23	25	25	83.3	32.0	1 - 3.1	2.05	0.6	0.8 - 3.9	2.44	0.96	1 -- 7	2.7	1.68
	0.50%	30	-	5	16	19	22	73.3	59.1	0.9 - 1.6	1.08	0.33		2.3	0		1	0
	0.75%	22	-	-	2	3	6	27.3	66.7		1.2	0	1 - 1.2	1.1	0.14			
	Control	28	20	24	24	24	24	85.7	16.7	1.3 - 4.9	2.79	1.07	0.8 - 5.5	2.55	1.32	1 -- 5	2.75	1.11

Table 29. Sub culturing response of embryo germinated seedlings in MS media									
Genotype	Teatment	No of plants cultured	Plumule length	Radicle length	No of leaves	% Mortality	Plant type before	Plant type after	
EC 318954	0.25%	4	3.36	3.66	5.66	25	R	R	
	0.50%	1	D	D	D	100	R	D	
	Control	10	6.55	4.1	7.3	0	R	R	
EC 329299	0.25%	4	3.3	4.1	3	75	R	R	
	0.50%	2	D	D	D	100	R	D	
	Control	13	4.4	4.23	4.88	30.76	R	R	
Wardan	0.25%	12	3.23	3.39	4.2	8.33	R	R	
	0.50%	1	2.4	2.9	2	0	R	R	
	0.75%	2	D	D	D	100	R	D	
	Control	20	5.11	4.5	6.42	15	R	R	
S= susceptible, R= resistant									

Table 30. Response of embryo germinated seedlings in rooting Media.									
			S	S	S	R	R	R	
Genotype	Treatment	No of plants cultured	Plumule length	Radicle length	No of leaves	Plumule length	Radicle length	No of leaves	% Mortality
EC 318954	0.25%	10	3.025		4.5	5.76	2.26	5.66	30
	0.50%	5	2.6		2	2.7	0.6	5	80
	Control	10				6.55	4.1	7.3	0
EC 329299	0.25%	1	D	D	D				100
	0.50%	1	D	D	D				100
	Control	13	4.4	4.23	4.89				30.76
Wardan	0.25%	6				2.3	2.29	4	50
	0.50%	5	1.73		2.66	2.4	5.55	5.5	0
	Control	20				5.11	4.5	6.42	15
S= susceptible, R= resistant									

Wardan

0.25% - Six plants were sub cultured of which 50% degenerated by 20th day. The average plumule length of the plants was 2.3 cm, the radicle length was 2.29 cm and the average numbers of leaves in the plants were 4.

0.50% - Five plants were sub cultured and no mortality was observed till 20th day. The average plumule length of the plants was 2.4 cm, the radicle length was 5.55 cm and the average number of leaves in the plants were 5.5.

Control - Twenty plants were sub cultured into RL media and by 20th day 15% of the plants degenerated. The average plumule length of the plants was 5.11 cm, the radicle length was 4.5 cm and the average number of leaves in the plants was 6.42.

H. Molecular characterization of selected genotypes

RAPD study was carried out using 30 decamer Random primers (Operon Technologies, Inc.) The polymerase chain reaction (PCR) was carried out in MJ research PTC 200 thermocycler. The PCR conditions were standardized and good amplification products obtained were considered in the study. Out of 30 decamer oligonucleotide primers used for amplification of Genomic DNA of 8 different genotypes of Berseem 7 (23.3%) did not react. The rest i.e. 23 primers (76.6%) generated one or more unambiguously scorable fragments. Depending on the primer genotypes combination and the amplification conditions used, the number of amplification products varied with primer N-20 producing maximum 14 distinct bands whereas primer OPR-06 and OPF-6 produced minimum 6 distinct bands. The 23 primers in total yielded 216 strong and easily scorable bands of which 71 bands were polymorphic (32.87%) and 145 bands (67.12%) were monomorphic (Table 31). The primer OPR-06 and AK-14 produced 6 and 9 distinct bands respectively all of which were monomorphic whereas the primer AB-10 produced 8 easily scorable bands all of which were polymorphic. Banding pattern obtained with different primers is presented here under (Table 32-34):

Primer OPE –12 revealed 11 distinct bands ranging approximately from 2Kb to 0.1Kb of which 9 bands were monomorphic whereas band No. 3 (MW. 1.45 Kb) and band No. 5 (MW. 1.0 Kb) were polymorphic and were absent in all the genotypes except ISH 8020B.

OPF-6 revealed 6 distinct and scorable bands from 2Kb to 0.1Kb of which band No. 2 of 1.0Kb was polymorphic and absent in all the genotypes except in ISH 8020B.

Primer OPG – 12 revealed the presence of 10 distinct bands ranging from 2Kb to 0.1Kb of which band No.1 (2Kb), 2 (1.4Kb), 3 (1.2 Kb) and 7(0.9Kb) were polymorphic. Band

No.1 and 7 were present only in the genotype EC 329299 and T45-1 whereas band No. 2 was absent in the genotype EC 329299, EC 407709 and ISH 8020B. The band No.3 was absent in the genotype Wardan, EC 407709 and T5-90I-1.

Primer OPH-9 revealed the presence of 11 distinct bands ranging from 2Kb to 0.1Kb of which only band No.5 and 9 were monomorphic whereas band No. 1(1.7Kb), 2(1.4Kb) and 3(1.2Kb) were absent only in the genotype Wardan and EC 407709. Band No.4 was absent only in the genotype ISH 8020B. Band No.6 and 8 were absent in the genotype EC 407709 and ISH 8020B. Band No.7 was present only in the genotype T 5-90I-1. Band No. 10 was absent in the genotype T 45-1-, EC 4017103 and ISH 8020B whereas band No.11 was absent in the genotype EC 407709 and ISH 8020B.

Primer OPQ-3 revealed the presence of 9 distinct bands of which band No. 1 and 2 (MW 1.2 Kb and 1.1 Kb) were absent in Wardan and EC 4017103 whereas band No. 5 (MW 0.8 Kb) was absent only in the genotype ISH 8020B. Band No. 8 was present only in the genotype Wardan and EC 4017103.

Primer OPN-6 revealed the presence of 9 distinct bands ranging from 2Kb to 0.1Kb of which only band No.2 (MW 1.0Kb) was polymorphic and absent in the genotypes EC 318954 and EC 4017103 only whereas the other bands were monomorphic.

Primer AE-01 revealed the presence of 9 distinct bands of which band No. 1 (MW 1.95Kb), 2(MW 1.5 Kb) and 9(0.3Kb) were polymorphic. Band No. 1 was absent in the genotype ISH 8020B whereas band No. 2 was present only in the ISH 8020B and absent in other genotypes. Band No. 9 was absent only in the genotype EC 318954.

Primer AE-03 revealed 8 easily scorable bands of which band No.1 (MW 1.95 Kb) and 3 (1.1 Kb) were present only in the genotype ISH 8020B and absent in all other genotypes whereas band No.2 (MW 1.45 Kb) was present in the genotypes EC 318954, EC 329299 and Wardan only.

Primer AH-9 revealed 11 distinct bands ranging from 2Kb to 0.1 Kb approximately, of which band No.1 (2Kb) and band No.3 (1.9 Kb) were polymorphic and all other bands were monomorphic in nature. Band No.1 was absent only in the genotype T 45-1 whereas band No.3 was present only in the genotype EC 407709, EC 4017103 and T 5-90I-1.

Primer OPQ-06 revealed 9 distinct bands of which only band No.1 (MW 1.7Kb) was polymorphic and present only in the genotypes T 5-90I-1 and ISH 8020B whereas all other bands were monomorphic.

Primer OPR-06 revealed the presence of 6 bands and all the bands were monomorphic. Primer AB – 05 revealed the presence of 12 distinct bands of which band No.2, 3, 4 and 12 were polymorphic and all other bands were monomorphic. The band No. 2(1.4 Kb) was present only in the genotype ISH 8020B, band No.3 (MW 1.2Kb) was absent only in the genotype Wardan, band No.4 (MW 1.1 Kb) was absent in the genotype EC 318954 and ISH 8020B whereas band No.12 (0.1Kb) was absent in the genotype EC 407709, EC 4017103 and T 5-90I-1.

Primer AB-10 revealed the presence of 8 distinct bands all of which were polymorphic. Thus this primer showed maximum polymorphism.

Primer AB-5 revealed the presence of 9 distinct bands of which band No. 1, 2, 5, 6 and 9 were polymorphic whereas the other bands were monomorphic. The band No.1 (MW 1.45 Kb) was present only in the genotype EC 318954 and EC 329299 only, band No.2 (MW 1.05 Kb) was present only in the genotype Wardan, band No.5 (MW 0.85 Kb) was present only in the genotype Wardan, EC 329299 and ISH 8020B only, band No.6 (MW 0.7 Kb) was present only in the genotype T 5-90I-1 whereas band No.9 (MW 0.55 Kb) was absent in the genotypes EC 407709, EC 4017103, T 5-90I-1 and ISH 8020B.

Primer AK – 14 revealed the presence of 9 distinct bands, all of which were monomorphic.

Primer U-01 revealed 10 distinct bands of which band No. 1, 2, 3, 7, 8 and 9 were polymorphic and the other bands were monomorphic. The band No.1 was present in all genotypes except EC 318954 and EC 329299, band No.2 (MW 1.6 Kb) was present only in the genotype EC 329299, band No.3(MW 0.95 Kb) was absent in the genotype EC 407709 and T 45-1, band No.7 (MW 0.65 Kb) was present in the genotype EC 329299, Wardan, EC 407709 and T 45-1 only, band No.8 (MW 0.5 Kb) was absent in the genotype EC 318954 and EC 329299 whereas band No.9 (MW 0.45 Kb) was present in the genotype EC 329299, Wardan and T45-1 only.

Primer P-9 revealed 8 distinct bands of which band No.1, 2 and 7 were polymorphic whereas all other bands were monomorphic. Band No.1 (MW. 2 Kb) was absent in the genotype EC 318954, band No.2 (MW 1.7 Kb) was present only in the genotype EC 329299 whereas band No.7 (MW 0.6 Kb) was present only in the genotype EC 329299, Wardan and T 45-1 only.

Primer H-15 revealed the presence of 9 distinct bands of which band No. 6 and 8 were polymorphic whereas the other bands were monomorphic in nature. The band No. 6 (MW 0.8 Kb) was present in the genotype Wardan and ISH 8020B only whereas band No.8 (MW 0.5 Kb) was present in the genotype Wardan only.

Primer E-16 revealed 9 distinct bands of which only band No.1 (MW 1.5 Kb) was polymorphic and present in the genotypes EC 318954, EC 329299 and T 45-1 only. The other bands were monomorphic.

Primer B-14 revealed 10 distinct bands of which band No. 1, 2, 3, 7 and 10 were polymorphic. The band No.1 (MW.≥ 2Kb) was present only in the genotypes EC 318954 and EC 329299, band No.2 (1.5Kb) was absent only in the genotype Wardan, band No.3 (MW 1.3 Kb) was absent in the genotype Wardan only, band No.7 (MW 0.7Kb) was absent in the genotypes EC 407709, T 45-1, EC 4017103 and ISH 8020B whereas band No.10 (MW 0.55Kb) was absent in the genotypes EC 407709, T 45-1, EC 4017103 and ISH 8020B. Thus the band 2 and 3 were specific to Wardan genotype whereas band 7 and 10 were specific to the genotypes EC 407709, T 45-1, EC 4017103 and ISH 8020B.

Primer V – 02 revealed 10 distinct band of which band No. 1, 4 and 9 showed polymorphism and the other bands were monomorphic. The band No.1 (MW> 2Kb) was absent in the genotype Wardan, EC 407709 and T 45-1, band No.4 (MW 1.0 Kb) was present only in the genotypes EC 318954 and EC 329299 whereas band No.9 (MW 0.6 Kb) was present only in the genotypes EC 329299, EC 407709 and T 45-1.

Primer N–20 revealed 14 distinct bands of which band No.2, 9, 11 and 13 showed polymorphic natures and the rest of the bands were monomorphic. The band No.2 (MW 1.5 Kb) was present only in the genotype EC 318954, band No.9 (MW. 0.7 Kb) was absent in the genotypes Wardan, EC 329299 and EC 318954, band No. 11 (MW. 0.6 Kb) was absent in the genotypes EC 318954, EC 4017103 and ISH 8020B whereas band No.13 (MW 0.5 Kb) was absent only in the genotype EC 407709.

Table 31. **List of primers and amplification products.**

S.No.	Name of Primer	Total No. of bands.	No. of monomorphic band	No. of polymorphic band
1.	OPE –12	11	9	2
2.	OPF – 6	6	5	1
3.	OPG - 12	10	6	4
4.	OPH – 9	11	2	9
5.	OPQ – 3	9	5	4
6.	OPN – 6	9	8	1
7.	AE – 01	9	8	1
8.	AE –03	8	5	3
9.	AH – 9	11	9	2
10.	OPQ – 06	9	7	2
11.	OPR – 06	6	6	-
12.	AB – 05	12	8	4
13.	AB – 10	8	1	7
14.	B – 5	9	4	5
15.	R – 8	9	7	2
16.	AK – 14	9	9	-
17.	U – 01	10	4	6
18.	P – 9	8	5	3
19.	H –15	9	7	2
20.	E –16	9	8	1
21.	B – 14	10	5	5
22.	V – 02	10	7	3
23.	N – 20	14	10	4
Total		216	145	71
Percentage			67.12%	32.87%

Table 32. RAPD Banding pattern in selected genotypes of Egyptian clover.																													
Primer	OPE-12																												
	Band No.										OPF-6						OPG-12												
Genotype	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10		
EC 318954	p	p		p		p	p	p	p	p	p	p		p	p	p	p		p	p	p	p	p		p	p	p		
EC 329299	p	p		p		p	p	p	p	p	p	p		p	p	p	p	p		p	p	p	p		p	p	p		
Wardan	p	p		p		p	p	p	p	p	p	p		p	p	p	p		p		p	p	p		p	p	p		
EC 407709	p	p		p		p	p	p	p	p	p	p		p	p	p	p				p	p	p		p	p	p		
T 45-1	p	p		p		p	p	p	p	p	p	p		p	p	p	p		p	p	p	p	p	p	p	p	p		
EC 4017103	p	p		p		p	p	p	p	p	p	p		p	p	p	p		p	p	p	p	p		p	p	p		
T 5-90I-1	p	p		p		p	p	p	p	p	p	p		p	p	p	p		p		p	p	p		p	p	p		
ISH 8020B	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p			p	p	p	p		p	p	p		
	OPH-9											OPQ-3									OPN - 6								
	1	2	3	4	5	6	7	8	9	10	11	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9
EC 318954	p	p	p	p	p	p		p	p	p	p	p	p	p	p		p	p		p	p		p	p	p	p	p	p	p
EC 329299	p	p	p	p	p	p		p	p	p	p	p	p	p	p		p	p		p	p	p	p	p	p	p	p	p	p
Wardan				p	p	p		p	p	p	p			p	p		p	p	p	p	p	p	p	p	p	p	p	p	p
EC 407709				p	p				p	p		p	p	p	p		p	p		p	p	p	p	p	p	p	p	p	p
T 45-1	p	p	p	p	p	p		p	p		p	p	p	p	p		p	p		p	p	p	p	p	p	p	p	p	p
EC 4017103	p	p	p	p	p	p		p	p		p			p	p		p	p	p	p	p		p	p	p	p	p	p	p
T 5-90I-1	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p	p		p	p	p	p	p	p	p	p	p	p
ISH 8020B	p	p	p		p				p			p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	p
	AE - 01									AE - 03								AH - 9											
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	10	11	
EC 318954	p		p	p	p	p	p	p			p		p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	
EC 329299	p		p	p	p	p	p	p	p		p		p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	
Wardan	p		p	p	p	p	p	p	p		p		p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	
EC 407709	p		p	p	p	p	p	p	p				p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	
T 45-1	p		p	p	p	p	p	p	p				p	p	p	p	p		p		p	p	p	p	p	p	p	p	
EC 4017103	p		p	p	p	p	p	p	p				p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	
T 5-90I-1	p		p	p	p	p	p	p	p				p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	
ISH 8020B		p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	

Table 33 RAPD Banding pattern in selected genotypes of Egyptian clover.

	OPQ - 06									OPR - 06						AB - 05											
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	1	2	3	4	5	6	7	8	9	10	11	12
EC 318954		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p		p	p	p	p	p	p	p	p
EC 329299		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	p
Wardan		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p			p	p	p	p	p	p	p	p	p
EC 407709		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	
T 45-1		p	p		p	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	p
Ec 4017103		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	
T 5-90I-1	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	
ISH 8020B	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p
	AB - 10								AB - 5									R- 08									
	1	2	3	4	5	6	7	8	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	
EC 318954		p	p	p	p	p	p	p	p		p	p			p	p	p	p	p	p	p	p	p	p	p		
EC 329299		p	p	p	p	p	p	p	p		p	p	p		p	p	p	p	p	p	p	p	p	p	p	p	
Wardan		p					p			p	p	p	p		p	p	p	p	p		p	p	p	p	p		
EC 407709		p					p				p	p			p	p		p	p	p	p	p	p	p	p		
T 45-1	p	p					p				p	p			p	p	p	p	p	p	p	p	p	p	p		
EC 4017103	p	p					p				p	p			p	p		p	p	p	p	p	p	p	p		
T 5-90I-1		p									p	p		p	p	p		p	p	p	p	p	p	p	p		
ISH 8020B							p				p	p	p		p	p	p	p	p	p	p	p	p	p	p		
	AK - 14									U - 01										P- 09							
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8
EC 318954	p	p	p	p	p	p	p	p	p			p	p	p	p				p			p	p	p	p		p
EC 329299	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p		p	p	p	p	p	p	p	p		p
Wardan	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p		p	p	p	p	p	p
EC 407709	p	p	p	p	p	p	p	p	p	p			p	p	p	p	p		p	p		p	p	p	p	p	p
T 45-1	p	p	p	p	p	p	p	p	p	p			p	p	p	p	p	p	p	p		p	p	p	p	p	p
EC 4017103	p	p	p	p	p	p	p	p	p	p		p	p	p	p		p		p	p		p	p	p	p		p
T 5-90I-1	p	p	p	p	p	p	p	p	p	p		p	p	p	p		p		p	p		p	p	p	p		p
ISH 8020B	p	p	p	p	p	p	p	p	p	p		p	p	p	p		p		p	p		p	p	p	p		p

Table 34. RAPD Banding pattern in selected genotypes of Egyptian clover.

	H - 15									E - 16									B - 14									
	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	1	2	3	4	5	6	7	8	9	10
EC 318954	p	p	p	p	p		p		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
EC 329299	p	p	p	p	p		p		p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p	p
Wardan	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p
EC 407709	p	p	p	p	p		p		p		p	p	p	p	p	p	p	p				p	p	p		p	p	
T 45-1	p	p	p	p	p		p		p	p	p	p	p	p	p	p	p	p		p	p	p	p	p		p	p	
EC 4017103	p	p	p	p	p		p		p		p	p	p	p	p	p	p	p		p	p	p	p	p		p	p	
T 5-90I-1	p	p	p	p	p		p		p		p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p
ISH 8020B	p	p	p	p	p	p	p		p		p	p	p	p	p	p	p	p		p	p	p	p	p		p	p	

	V - 02										N - 20													
	1	2	3	4	5	6	7	8	9	10	1	2	3	4	5	6	7	8	9	10	11	12	13	14
EC 318954	p	p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p		p		p	p	p
EC 329299	p	p	p	p	p	p	p	p	p	p	p		p	p	p	p	p	p		p	p	p	p	p
Wardan		p	p		p	p	p	p		p	p		p	p	p	p	p	p		p	p	p	p	p
EC 407709		p	p		p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	p	p	p
T 45-1		p	p		p	p	p	p	p	p	p		p	p	p	p	p	p	p	p	p	p		p
EC 4017103	p	p	p		p	p	p	p		p	p		p	p	p	p	p	p	p	p		p	p	p
T 5-90I-1	p	p	p		p	p	p	p		p	p		p	p	p	p	p	p	p	p	p	p	p	p
ISH 8020B	p	p	p		p	p	p	p		p	p		p	p	p	p	p	p	p	p		p	p	p

Plate 1

In vitro **seedling vigour of Egyptian clover genotypes *vis-à-vis* normal condition (20 day old seedlings)**

Fig A to D.	EC 318954	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig E to H.	EC 329299	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig I to L.	Wardan	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig M to P.	EC 400976	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig Q to T.	EC 508311	A. Control. B. 0.25% C. 0.50% D. 0.75%

Plate 1

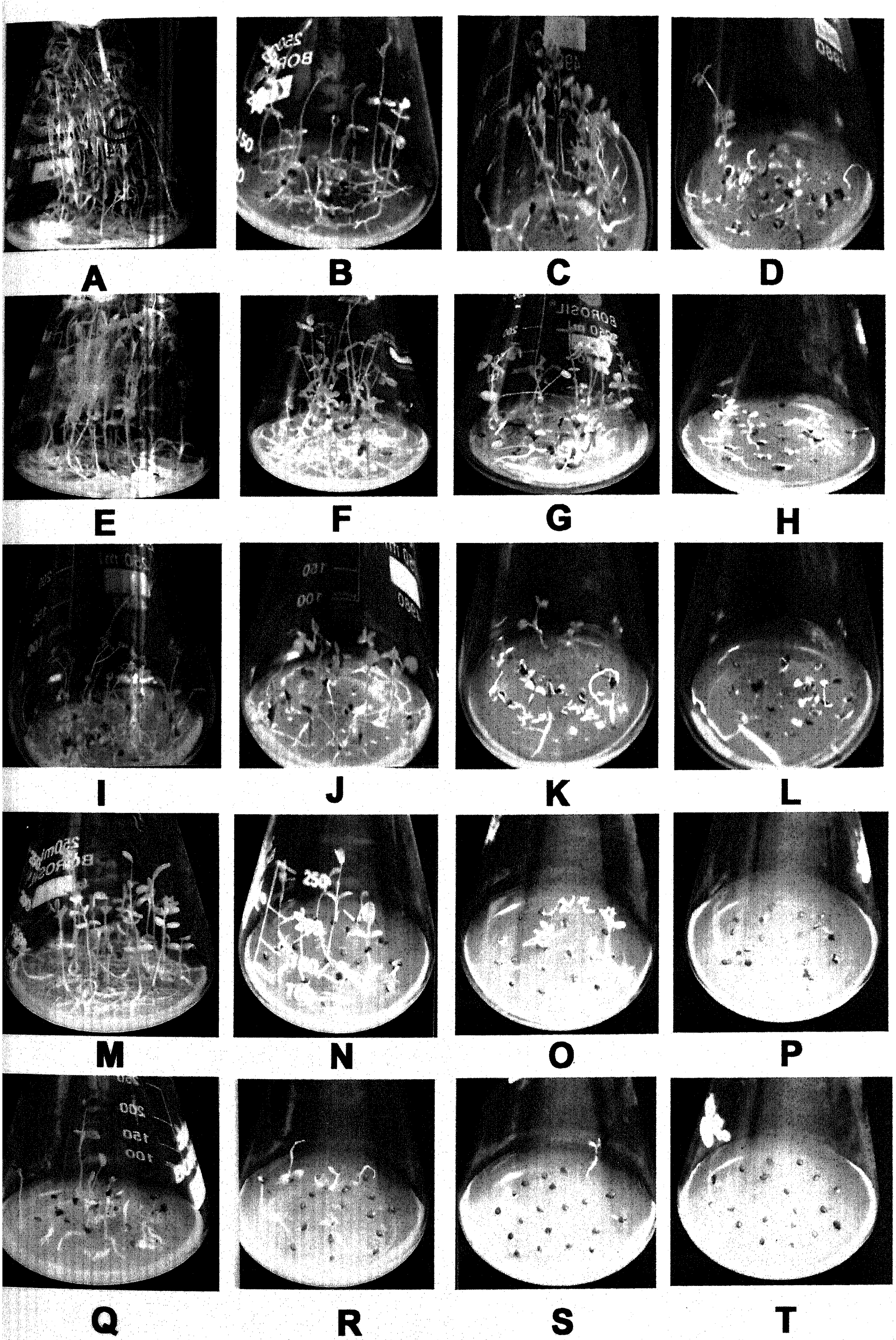


Plate 2

***In vitro* seedling vigour of Egyptian clover genotypes *vis-à-vis* normal condition (20 day old seedlings)**

Fig A to D.	EC 407709	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig E to H.	ISH 32/8/1	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig I to L.	ES 99	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig M to P.	Penta 99-1	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig Q to T.	Raj Bundi	A. Control. B. 0.25% C. 0.50% D. 0.75%
Fig U to X.	Penta 99	A. Control. B. 0.25% C. 0.50% D. 0.75%

Plate 3

***In vitro* seedling vigour of Egyptian clover genotypes *vis-à-vis* normal condition (20 day old seedlings)**

Fig A to D. ISH 34/49 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig E to H. ISH 34/41 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig I to L. 34/11 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig M to P. T 5-90I-1 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig Q to T. T 44-4 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig U to X. T 45-1 A. Control. B. 0.25% C. 0.50% D. 0.75%

Plate 3

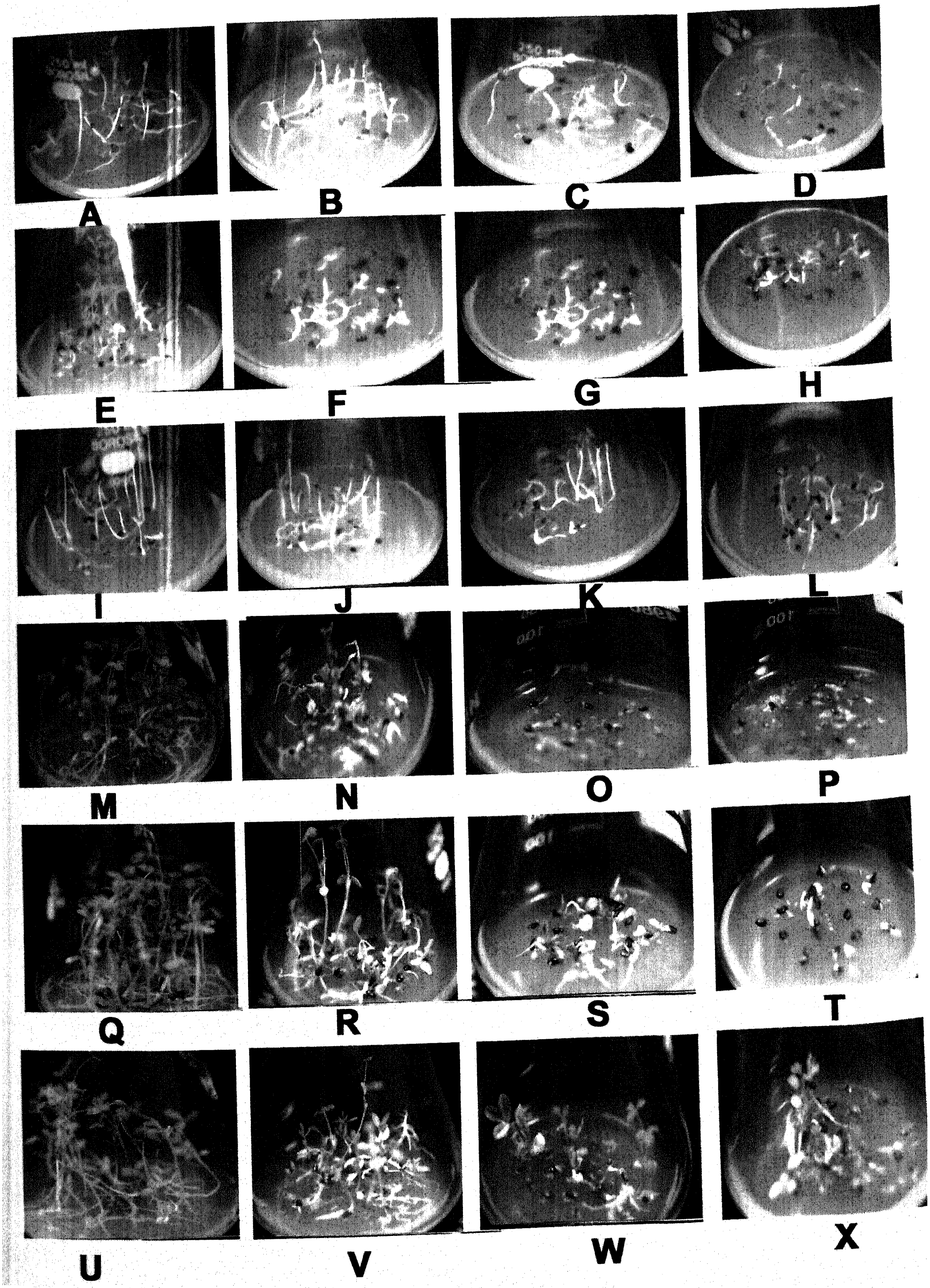


Plate 4

***In vitro* plant growth of Egyptian clover genotypes under saline *vis-à-vis* normal condition (45 day old plants)**

Fig A to D. EC 318954 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig E to H. EC 329299 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig I to L. Multi-98-45 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig M to P. Raj 49/50 A. Control. B. 0.25% C. 0.50% D. 0.75%

Fig Q to T. ISH 34/5/1 A. Control. B. 0.25% C. 0.50% D. 0.75%

Plate 4

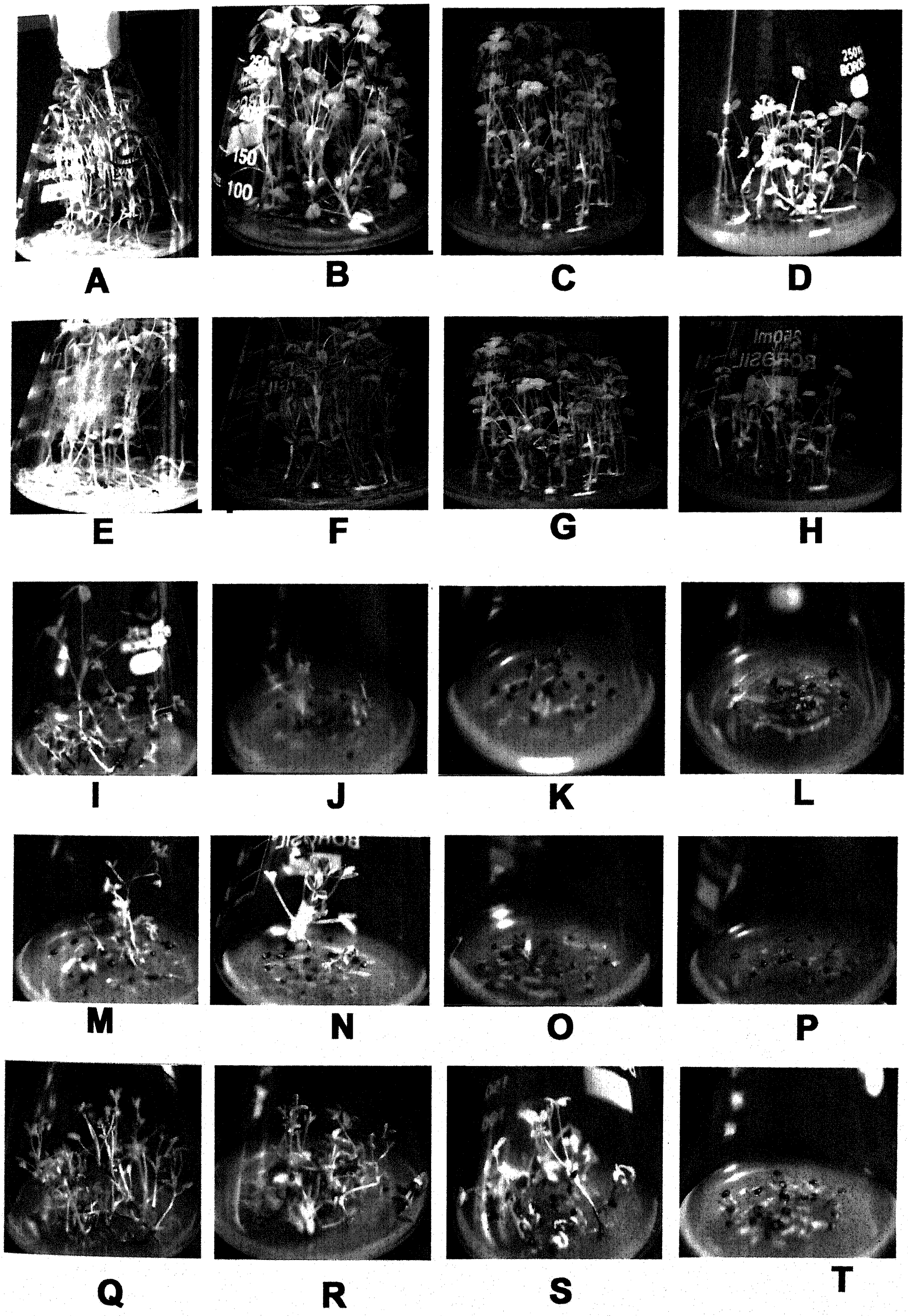


Plate 5

Peroxidase banding pattern in Egyptian clover genotypes growing *in vitro* under saline vis-à-vis normal condition.

Fig A.. (L to R).. **Samples 1 to 7 - EC 407709** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4. 0.50%R, 5. 0.75%S 6. 0.75%R and 7.control. **Sample 8 to 12 - EC 400976** 8. 0.25%S, 9. 0.25%R, 10. 0.50%S, 11. 0.75%S, 12. Control and 13. Test sample.
Fig B. (R to L). **Sample 1 to 4 - ISH 34/41** 1. 0.25%S, 2. 0.50%S, 3. 0.75%S, 4. Control. **Sample 5 to 10. ISH 34/11** 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. 0.50%R, 9. 0.75%S, 10. Control and 13.Test sample.
Fig C. (R to L). **Sample 1 to 3 - EC 4017103** 1. 0.25%S, 2. 0.50%S, 3. Control. Sample 4 to 7 EC 400977 4. 025%S, 5. 0.50%S, 6. 0.75%S
7. Control.**Sample 8 to 11 - EC 401711** 8. 0.25%S 9. 0.25%R 10. 0.50%S 11. Control and 12. Test sample.
Fig D. (L to R). **Sample 1 to 4 - Wardan S2** 1. 0.25%S, 2. 0.50%S, 3. 0.75%S, 4. Control. **Sample 5 to 8 - ISH 26/50/7** 5. 0.25%S, 6. 0.25%R, 7. Control, 8. 0.75%S and 9. Test sample.
Fig E. (L to R). **Sample 1 to 4 - EC 508311** 1. 0.25%S, 2. 0.50%S, 3 and 4. Control. **Sample 5 to 11 - ISH 32/34/1** 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. 0.50%R, 9. 0.75%S, 10. Control and 11. Test sample.
Fig F. (L to R).**Sample 1 to 4 - Multi-98-45** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4. Control. Sample 5 to 9. ISH 34/5/1 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. 0.75%S, 9. Control and10. Test sample

Esterase banding pattern in Egyptian clover genotypes growing under sand culture condition

Fig G. (L to R). **Sample 1 to 4 - EC 329299** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 5 to 8 - EC 318954** 5. 0.50%, 6. 0.75%, 7. 1%, 8. Control. **Sample 9 to 12 - T 45-1** 0.9. 50%, 10. 0.75%, 11. 1% and 12. Control.
Fig H. (L to R). **Sample 1 to 4 - EC 407709** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 5 to 8 - ISH 8020B** 5. 0.50%, 6. 0.75%, 7. 1% and 8. Control.

SOD banding pattern in Egyptian clover genotypes growing under sand culture condition

Fig I. (R to L). **Sample 1 to 4 - EC 329299** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 5 to 8 - EC 318954** 5. 0.50%, 6. 0.75%, 7. 1%, 8. Control. **Sample 9 to 12 - T 45-1** 9. 0.50%, 10. 0.75%, 11. 1% and 12. Control.
Fig J. (L to R). **Sample 1 to 4 - EC 407709** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 5 to 8 - ISH 8020B** 5. 0.50%, 6. 0.75%, 7. 1% and 8. Control.

***In vitro* plant growth (45 days) under saline vis-à-vis normal condition.**

Fig K. (R to L) **ISH 8020B** 0.25% S and R, 0.50% S and R, 0.75% S and R and control.
Fig L. (R to L) **ISH 8020Y** 0.25% S and R, 0.50% S and R, 0.75% S and control.
Fig M. (R to L) **ISH 5050B** 0.25% S and R, 0.50% S and R, 0.75% S and control.
Fig N. (R to L) **ISH 5050Y** 0.25% R and S, 0.50% S and R, 0.75% S, control and test sample.
Fig O. (R to L) **ISH 34/5/1** 0.75% S, 0.50% S, 0.25% R and control.

Plate 5

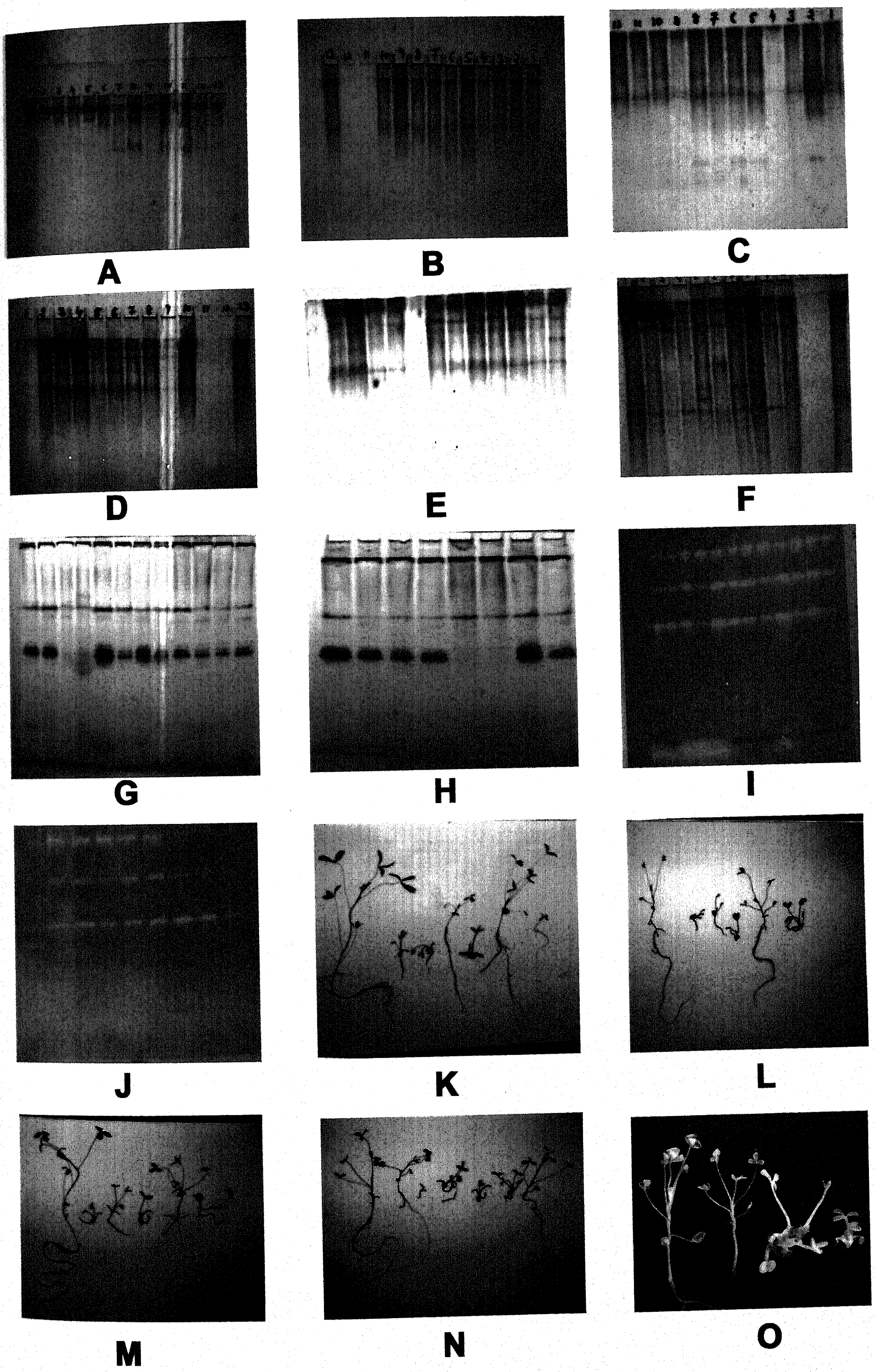


Plate 6

Esterase banding pattern in Egyptian clover genotypes growing in saline vis-à-vis normal condition.

Fig A. (L to R). **Sample 1 to 6 - EC 329299** 1. 0.25%R, 2. 0.50%S, 3. 0.50%R, 4. 0.75%S, 5. 0.75%R, 6. Control. **Sample 7 to 12 - EC 318954** 7. 0.25%R, 8. 0.50%S, 9. 0.50%R, 10. 0.75%S, 11. 0.75%R, 12. Control and 13. Test sample.

Fig B. (L to R).**Sample 1 to 6 - Wardan** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4. 0.50%R, 5. 0.75%S and 6. Control.

Fig C. (R to L). **Sample 1 to 7 - EC 407709** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4. 0.50%R, 5. 0.75%S, 6. 0.75%R, 7. Control. **Sample 8 to 12 - EC 400976** 8. 0.25%S, 9. 0.25%R, 10. 0.50%S, 11. 0.75%S, 12. Control and 13. Test sample.

Fig D. (R to L). **Sample 1 to 3 - EC 508311** 1. 0.25%S, 2. 0.50%S, 3. Control and 4. Test sample.

Fig E. (R to L). **Sample 1 to 3 - EC 4017103** 1. 0.25%S, 2. 0.50%S, 3. Control. **Sample 4 to 7 - EC 400977** 4. 0.25%S, 5. 0.50%S, 6. 0.75%S, 7. Control. **Sample 8 to 11 - EC 401711** 8. 0.25%S, 9. 0.25%R, 10. 0.50%S, 11.Control and 12. Test sample.

Fig F. (L to R).**Sample 1 to 4 - ISH 34/41** 1. 0.25%S, 2. 0.50%S, 3. 0.75%S, 4. Control. **Sample 5 to 10 - ISH 34/11** 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. 0.50%R, 9. 0.75%S, 10. Control and 11.Test sample.

Fig G. (L to R).**Sample 1 to 4 - Multi-98-45** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4.Control. **Sample 5 to 10 - ISH 34/5/1** 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. 0.75%S, 9 and 10. Control and 11. Test sample.

Fig H. (L to R).**Sample 1 to 4 - Wardan S2** 1. 0.25%S, 2. 0.50%S, 3. 0.75%S, 4. Control. **Sample 5 to 9 - ISH 26/50/7** 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. 0.75%S, 9. Control. **Sample 10 to 12 - ISH 32/34/1** 10. 0.25%S, 11. 0.25%R, 12. Control. and 13. Test sample.

Fig I. (L to R).**Sample 1 to 6 - ISH 32/34/1** 1. 0.25%S, 2, 0.25%R, 3. 0.50%S, 4. 0.50%R, 5. 0.75%S, 6. Control and 7.Test sample.

Fig J. (L to R).**Sample 1 to 5 - T 5-90I-1** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4. 0.50%R, 5. Control. **Sample 6 to 10 - T 45-1** 6. 0.25%S, 7. 0.25%R, 8. 0.50%S, 9. 0.50%R, 10. Control .**Sample 11 to 12 - T 44-4** 11. 0.25%S, 12. 0.25%R and 13. Test sample.

Fig K. (R to L).**Sample 1 to 2 - ES 99** 1. 0.25%S, 2. Control. **Sample 3 to 5 - ISH 32/8/1** 3. 0.25%S, 4. 0.50%S, 5. Control. **Sample 6 to 9 - ISH 5050Y** 6. 0.25%S, 7. 0.25%R, 8. 0.50%S, 9. Control and 10. Test sample.

Fig L. (R to L).**Sample 1 to 4 - ISH 5050B** 1. 0.25%S, 2. 0.25%R, 3. 0.50%S, 4. Control. **Sample 5 to 8 - ISH 8020B** 5. 0.25%S, 6. 0.25%R, 7. 0.50%S, 8. Control.**Sample 9 to 12 - ISH 8020Y** 9. 0.25%S, 10. 0.25%R, 11. 0.50%S, 12. Control and 13. Test sample.

Plate 6

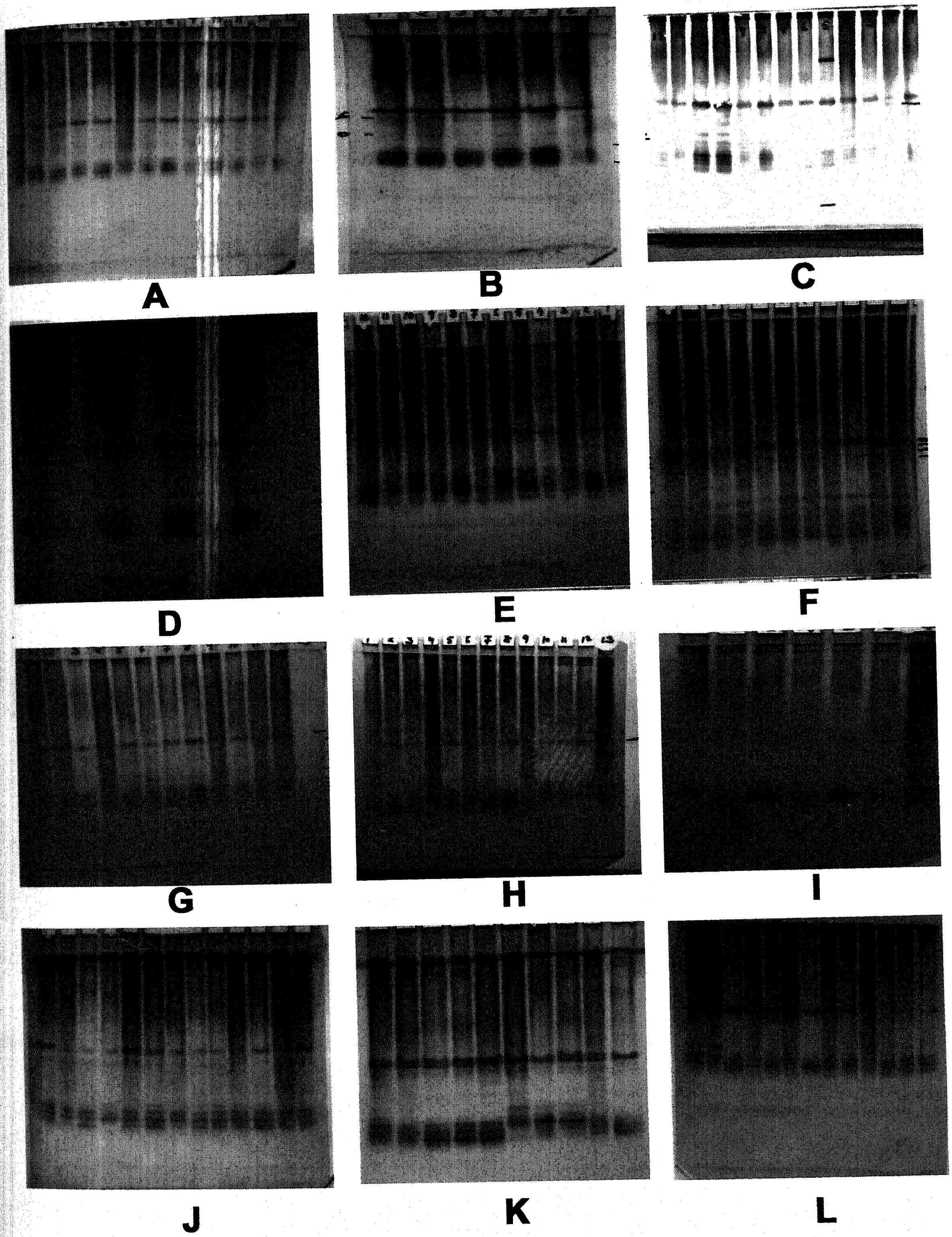


Plate 7

***In vitro* callusing response of Egyptian clover genotypes under saline *vis-à-vis* normal condition.**

Fig A. EC 329299 Explant – Hypocotyl 0.25%.

Fig B. EC 329299 Exlpant – Hypocotyl 0.50%.

Fig C. EC 329299 Exlpant – Hypocotyl 0.75%.

Fig D. EC 329299 Explant – Hypocotyl 1%.

Fig E. EC 329299 Explant - Hypocotyl 1.25%.

Fig F. EC 329299 Explant - Hypocotyl Control.

Fig G. EC 329299 Explant – Petiole 0.25%.

Fig H. EC 329299 Explant – Petiole 0.50%.

Fig I. EC 329299 Explant – Petiole 0.75%.

Fig J. EC 329299 Explant - Petiole 1%.

Fig K. EC 329299 Explant – Petiole 1.25%.

Fig L. EC 329299 Explant – Petiole Control.

Fig L to R EC 329299, Wardan and EC 318954 Explant – Petiole (0.25% to 0.50%).

Fig N. L to R EC 318954, EC 329299 and Wardan Explant – Hypocotyl (0.50% to regenerating media).

Fig O. L to R EC 318954, EC 329299 and Wardan Explant – Hypocotyl (0.25% to 0.50% salinity).

Plate 7

A

B

C

D

E

F

G

H

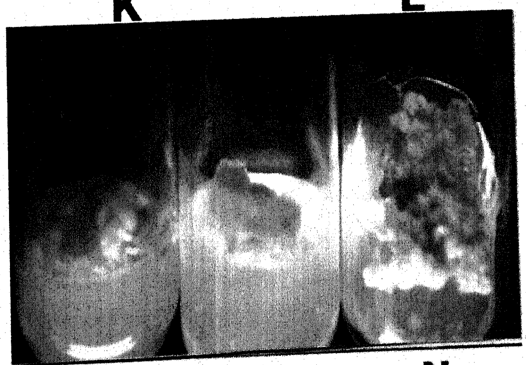
I

J

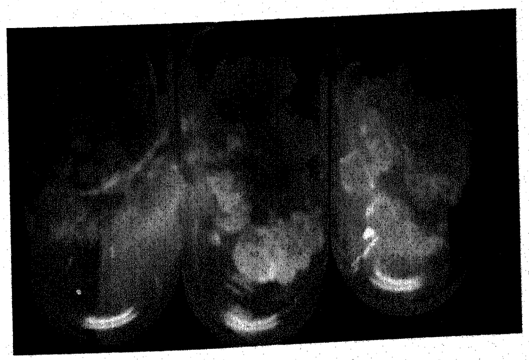
K

L

M



N



O

Plate 8

Embryo culture response of Egyptian clover genotypes in saline *vis-à-vis* normal condition.

Fig A. Left to right mature embryos EC 318954, EC 329299 and Wardan.
Fig B. EC 318954 0.25% 1/2MS basal media.
Fig C. EC 318954 0.50% 1/2MS basal media.
Fig D. EC 318954 0.75% 1/2MS basal media.
Fig E. EC 318954 control 1/2MS basal media.
Fig F. EC 318954 0.25% MS basal media.
Fig G. EC 318954 0.50% MS basal media.
Fig H. EC 318954 0.75% MS basal media.
Fig I. EC 318954 control MS basal media.
Fig J. (L to R) Wardan 0.25%, 0.50%, 0.75% and control MS basal media.
Fig K. (L to R) EC 329299 0.25%, 0.50%, 0.75% and control MS basal media.
Fig L. (L to R) EC 318954 0.25%, 0.50%, 0.75% and control MS basal media.
Fig M. (L to R)Sub culturing response Wardan 0.25%, 0.50%, 0.75% and control.
Fig N. Sub culturing response in rooting media EC 318954 0.25%.
Fig O. Embryo germinated plant of Wardan in pot.

Plate 8

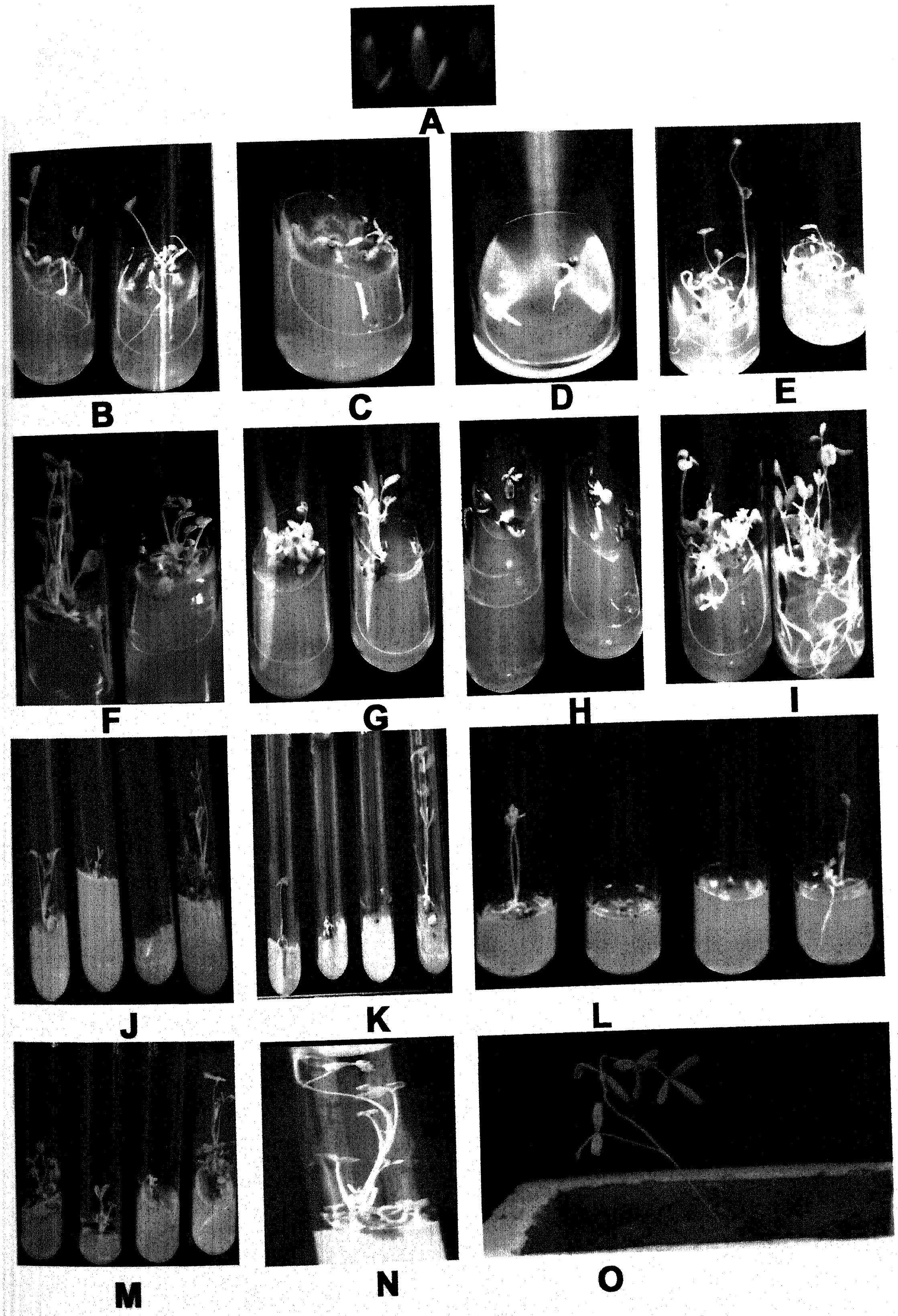


Plate 9

Protein profile in Egyptian clover genotypes growing in saline *vis-à-vis* normal condition

Fig A. SDS banding pattern. (L to R).**Sample 1 to 4 - EC 329299** 1. 0.50%, 2. 0.75%, 3. 1% 4. Control. **Sample 5 to 8 - EC 318954** 5. 0.50%, 6. 0.75%, 7. 1%, 8. Control. **Sample 9 to 10 - T 45-1** 9. 0.50%, 10. 0.75% and 11. Molecular weight marker.

Fig B. SDS banding pattern. (L to R). **Sample 1 to 2 - T 45-1** 1. 1%, 2. Control. **Sample 3 to 6 - EC 407709** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 7 to 10 - ISH 8020B** 7. 0.50%, 8. 0.75%, 9. 1%, 10. Control and 11. Molecular weight marker.

Fig C. Native PAGE Protein profile. (R to L). **Sample 1 to 4 - EC 329299** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 5 to 8 - EC 318954** 5. 0.50%, 6. 0.75%, 7. 1%, 8. Control. **Sample 9 to 12 - T 45-1** 9. 0.50% 10. 0.75%, 11. 1%, 12. Control.

Fig D. Native PAGE Protein profile. (R to L). **Sample 1 to 4 - EC 407709** 1. 0.50%, 2. 0.75%, 3. 1%, 4. Control. **Sample 5 to 8 - ISH 8020B** 5. 0.50%, 6. 0.75%, 7. 1%, 8. Control.

Fig E. Native PAGE Protein profile. (L to R). **Sample 1 to 5 - Wardan** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. 1%, 5. Control. **Sample 6 to 10 - EC 407709** 6. 0.25%, 7. 0.50%, 8. 0.75%, 9. 1% and 10. Control

Fig F. Native PAGE Protein profile. (L to R). **Sample 1 to 5 - T 45-1** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. 1%, 5. Control. **Sample 6 to 10 - ISH 8020B** 6. 0.25%, 7. 0.50%, 8. 0.75%, 9. 1% and 10. Control.

Fig G. Native PAGE Protein profile. (L to R). **Sample 1 to 4 - EC 318954** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. Control. **Sample 5 to 8 - EC 329299** 5. 0.25%, 2. 0.50%, 3. 0.75% and 8. Control.

Fig H. Native PAGE Protein profile. (L to R). **Sample 1 to 4 - EC 4017103** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. Control. **Sample 5 to 8 - T 5-90I-1** 5. 0.25%, 2. 0.50%, 3. 0.75% and 8. Control.

Fig I. SDS banding pattern. (L to R). **Sample 1 to 5 - Wardan** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. 1%, 5. Control. **Sample 6 to 10 - EC 407709** 6. 0.25%, 7. 0.50%, 8. 0.75%, 9. 1% 10. Control. Sample 11. Molecular weight marker.

Fig J. SDS banding pattern. (L to R). **Sample 1 to 5 - T 45-1** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. 1%, 5. Control. **Sample 6.** Molecular weight marker. **Sample 7 to 11 - ISH 8020B** 7. 0.25%, 8. 0.50%, 9. 0.75%, 10. 1% and 11. Control

Fig K SDS banding pattern. (L to R). **Sample 1 to 4 - EC 318954** 1. 0.25%, 2. 0.50%, 3. 0.75%,.4. Control. **Sample 5.** Molecular weight marker. **Sample 6 to 9 - EC 329299** 6. 0.25%, 7. 0.50%, 8. 0.75% and 9. Control.

Fig L. SDS banding pattern. (L to R). **Sample 1 to 4 - EC 4017103** 1. 0.25%, 2. 0.50%, 3. 0.75%, 4. Control. **Sample 5 to 8 - T 5-90I-1** 5. 0.25%, 6. 0.50%, 7. 0.75% 8. Control. **Sample 9.** Molecular weight marker.

Plate 9

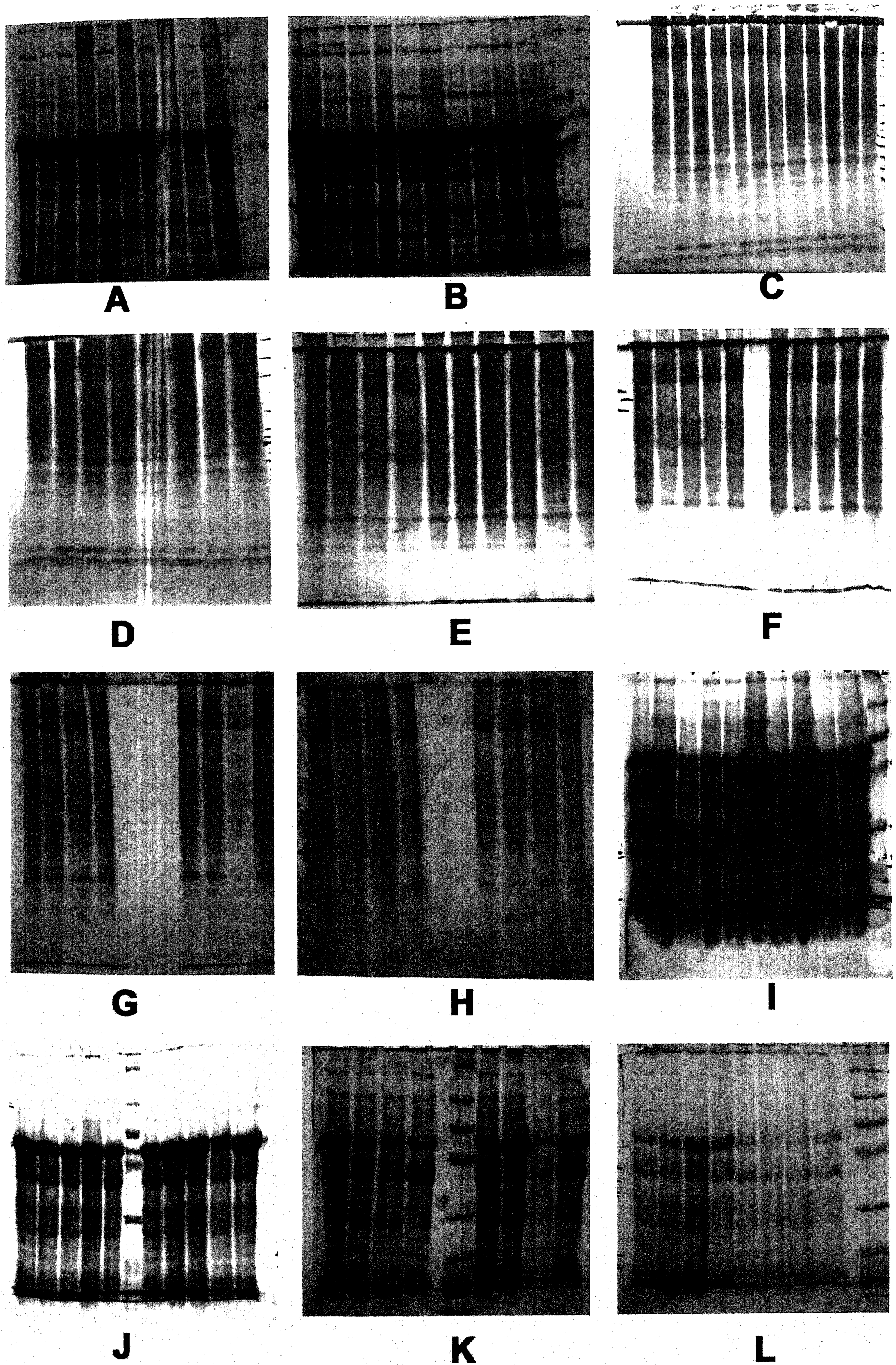


Plate 10

Effect of secondary salinization on selected Egyptian clover genotypes in pot culture condition (60 day old).

Fig A. (L to R). Pot 1 to 4. EC 318954 **1.** 1%, 2. 0.75%, 3. 0.50% and 4. Control.

Fig B. (L to R). Pot 1 to 4. ISH 8020B **1.** 1%, 2. 0.75%, 3. 0.50% and 4. Control.

Fig C. (L to R). Pot 1 to 4. T 45-1 1. 1%, 2. 0.75%, 3. 0.50% and 4. Control

Fig D. (L to R). Pot 1 to 4. EC 407709 1. 1%, 2. 0.75%, 3. 0.50% and 4. Control.

Fig E. (L to R). Pot 1 to 4. EC 329299 **1.** 1%, 2. 0.75%, 3. 0.50% and 4. Control.

Fig F. Individual plants (L to R). T 45-1 **1.** Control, 2. 0.50%, 3. 0.75% and 4. 1%.

Fig G. Individual plants (L to R). EC 329299 **1.** 1%, 2. 0.75%, 3. 0.50% and 4.Control.

Fig H. Individual plants (L to R). EC 318954 **1.** Control, 2. 0.50%, 3. 0.75% and 4.1%.

Fig I. Individual plants (L to R). ISH 8020B **1.** Control, 2. 0.50%, 3. 0.75% and 4. 1%

Fig J. Individual plants (L to R). EC 407709 **1.** Control, 2. 0.50%, 3. 0.75% and 4. 1%

Fig K. Roots of plants (L to R). EC 318954 **1.** Control, 2. 0.50%, 3. 0.75% and 4. 1%.

Fig L. Roots of plants (L to R). T 45-1 1. Control, 2. 0.50%, 3. 0.75% and 4. 1%.

Fig M. Roots of plants (L to R). EC 407709 **1.** Control, 2. 0.50%, 3. 0.75% and 4. 1%.

Fig N. Roots of plants (L to R). ISH 8020B **1.** 1%, 2. 0.75%, 3. 0.50% and 4. Control.

Plate 10

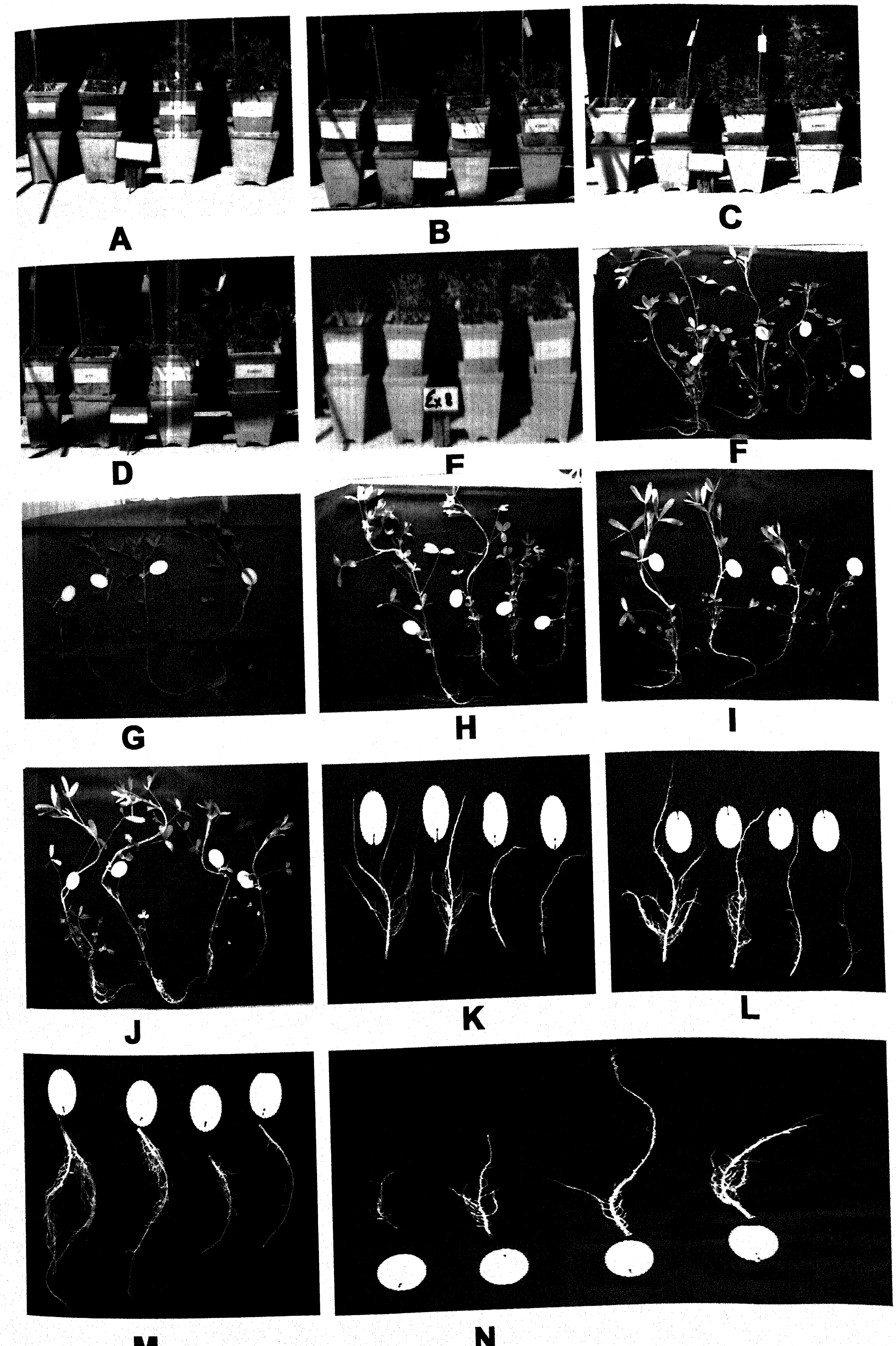


Plate 11

Molecular characterizations of Egyptian clover genotypes using RAPD analysis.

Fig A.

Top row

PCR pattern with **primers AB-10** (Lane B to I), **AB-5** (Lane J to Q), **R-8** (Lane R to Y). Sample sequence with each primer (L to R). EC 318954, EC 329299, Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and ISH 8020B. Lane A- DNA ladder 10 to 100 bp.

Bottom row

PCR pattern with primers **AK-14** (Lane B to I), **U-01** (Lane J to Q), **P-9** (Lane R to Y). Sample sequence with each primer (L to R). EC 318954, EC 329299, Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

Fig B.

Top Row

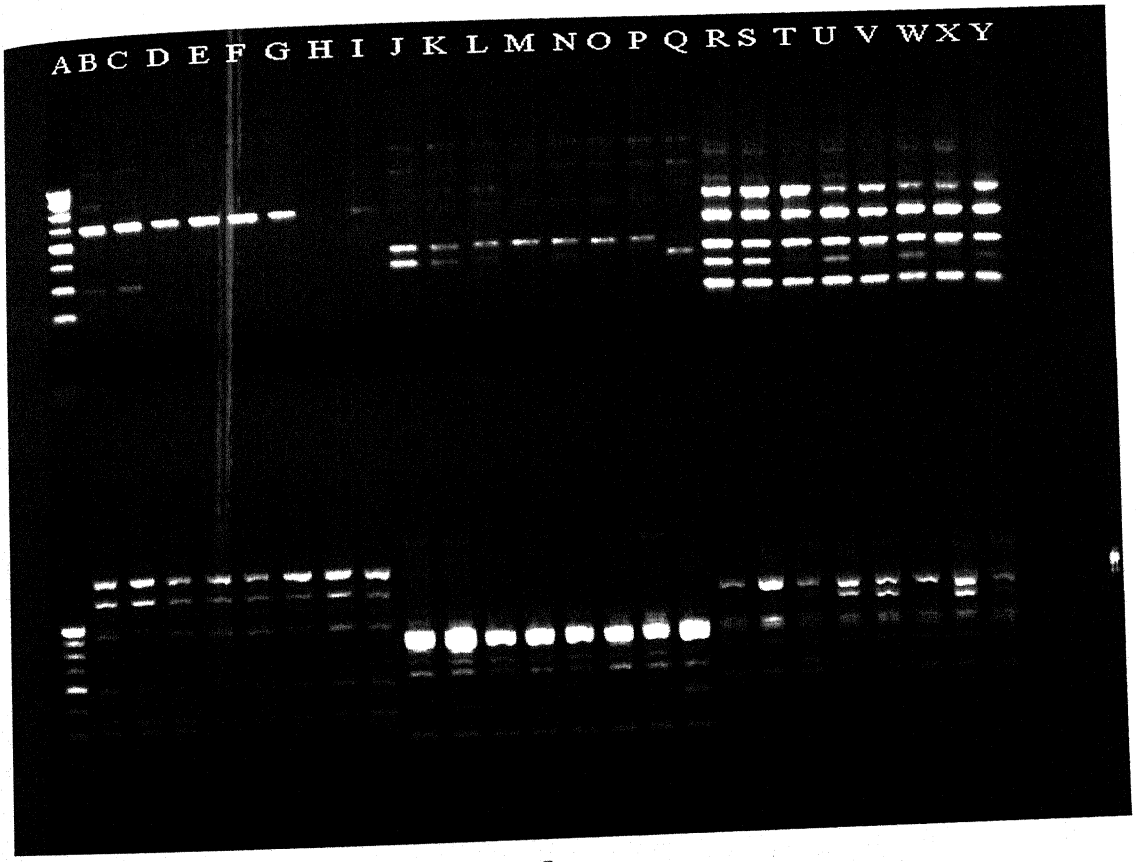
PCR pattern with primers **H-15** (Lane B to I), **E-16** (Lane J to Q) **G-20** (Lane R to Y),

Sample sequence with each primer (L to R). EC 318954, EC 329299, Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

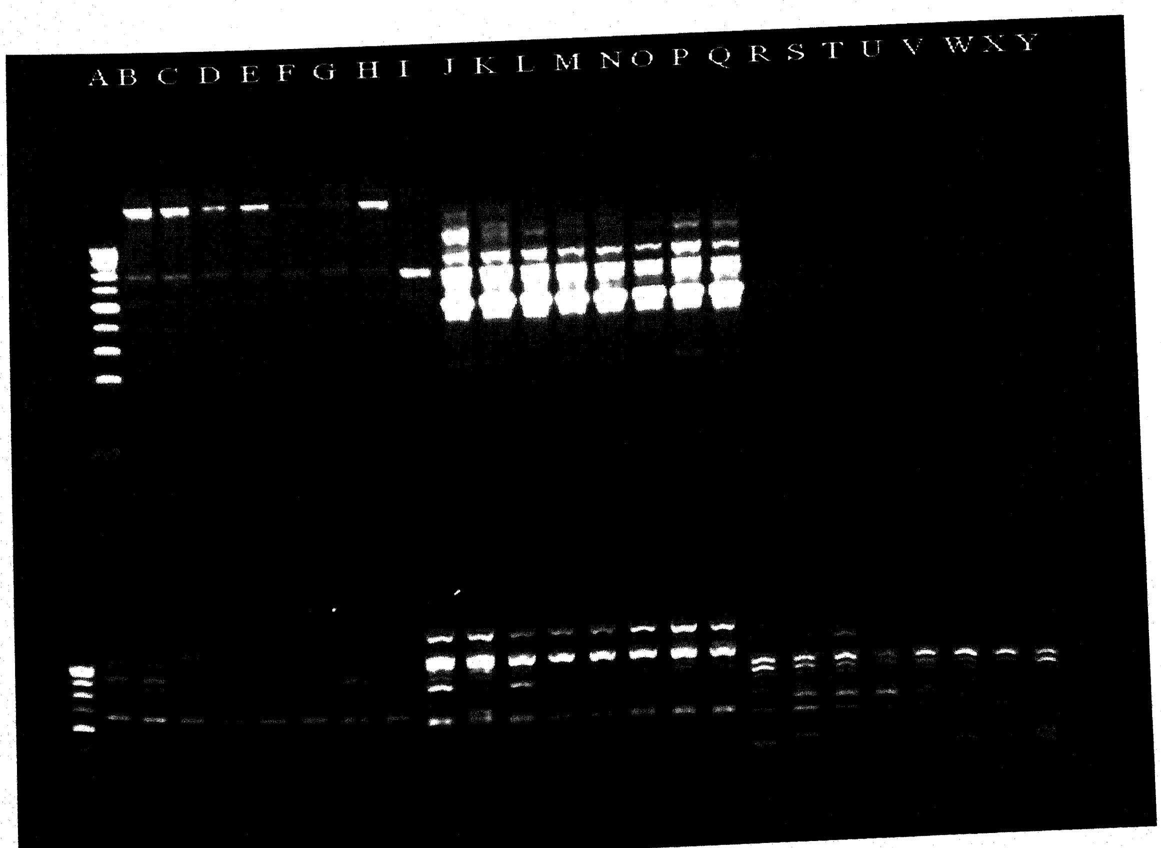
Bottom Row

PCR pattern with primers **B14** (Lane B to I), **V-20** (Lane J to Q) and **N-20** (Lane R to Y), Sample sequence with each primer (L to R). EC 318954, EC 329299, Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

Plate 11


A B C D E F G H I J K L M N O P Q R S T U V W X Y

A


A B C D E F G H I J K L M N O P Q R S T U V W X Y

B

Plate 12

Molecular characterizations of Egyptian clover genotypes using RAPD analysis.

Fig A.

<u>Top Row</u>

PCR pattern with primers **AE-01** (Lane B to I), **AE-03** (Lane J to Q) and **AH-9** (Lane R to Y) Sample sequence with each primer (L to R). EC 318954, EC 329299, Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

<u>Bottom Row</u>

PCR pattern with primers **OPQ-06** (Lane B to I), **OPR-06** (Lane J to Q) and **AB-5** (Lane R to Y). Sample sequence with each primer (L to R). EC 318954, 329299. Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

Fig B.

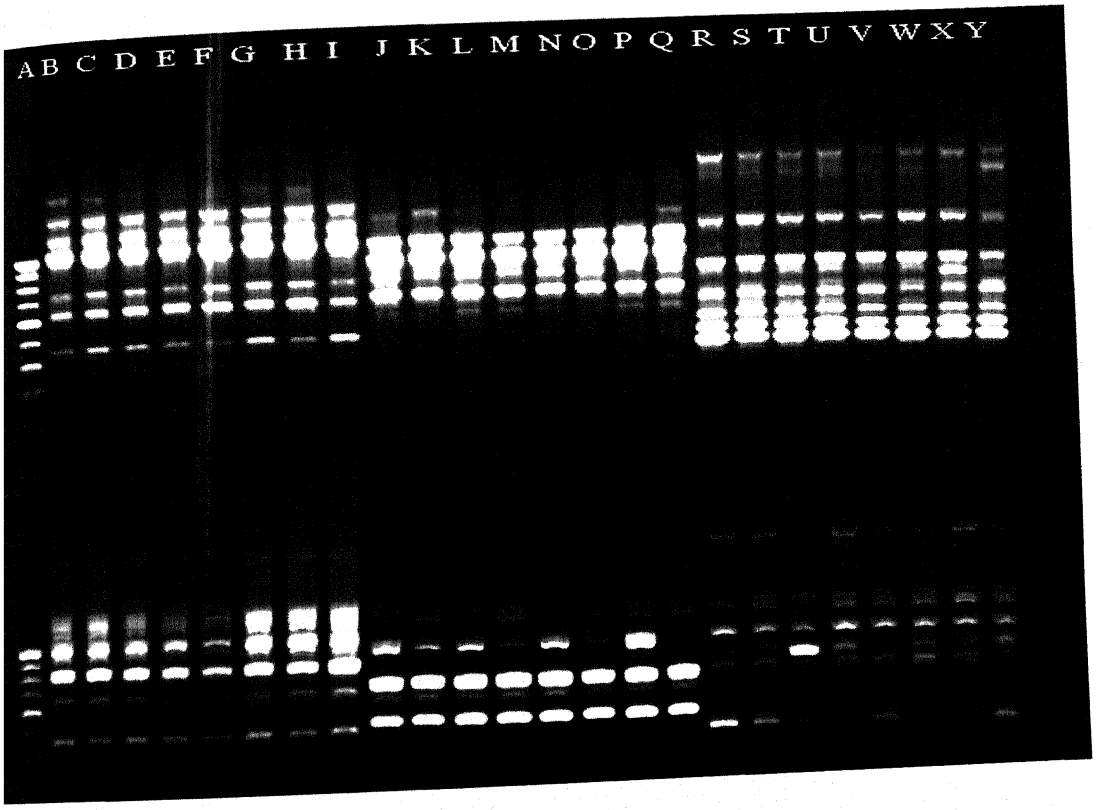
<u>Top Row</u>

PCR pattern with primers **OPE-12** (Lane B to I), **OPF-6** (Lane J to Q), **OPG-125** (Lane R to Y), Sample sequence with each primer (L to R). EC 318954, 329299. Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and. ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

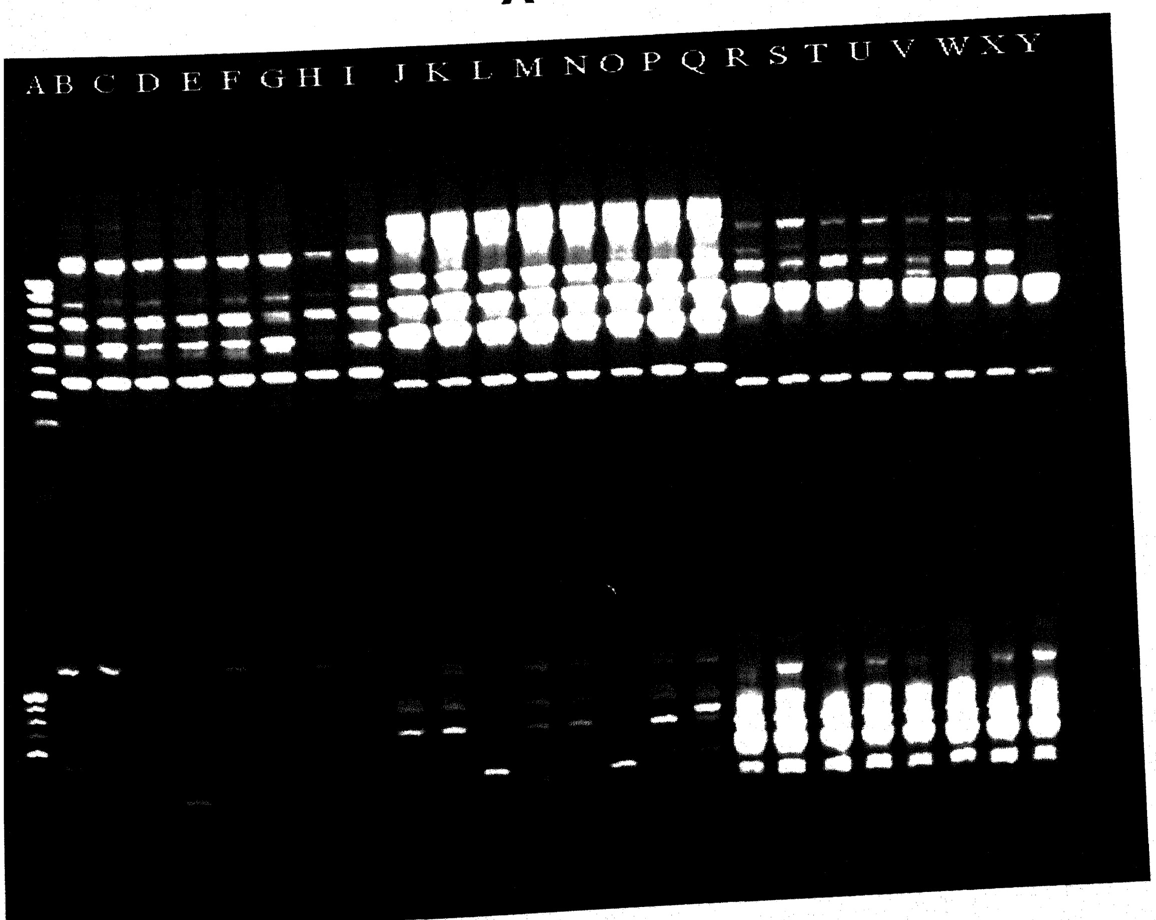
<u>Bottom Row</u>

PCR pattern with primers **OPH-9** (Lane B to I), **OPQ-3** (Lane J to Q) and **OPN-6** (Lane R to Y). Sample sequence with each primer (L to R). EC 318954, 329299. Wardan, EC 407709, T 45-1, EC 4017103, T 5-90I-1 and. ISH 8020B. Lane A-DNA ladder 10 to 100 bp.

Plate 12


A B C D E F G H I J K L M N O P Q R S T U V W X Y

A


A B C D E F G H I J K L M N O P Q R S T U V W X Y

B

DISCUSSION

DISCUSSION

The discussion on the present work is grouped in following heads:

A. *In vitro* germination, survival, seedling vigour and growth.

B. Biochemical studies.

C. Effect of secondary salinization.

D. *In vitro* callusing and embryo culture response.

E. Molecular characterization of genotypes.

D. Identification of genotypes.

A. *In vitro* germination, survival, seedling vigour and growth.

A.1 Germination

Germination was adversely affected with increasing level of salinity. The effect was manifested in reduced and delay in germination. The degree of adverse effect varied with genotypes and salinity levels. Genotypes like EC 318954 showed at par germination to the control conditions at 0.25% and 0.50% salinity. Genotypes like EC 407709, ISH 34/11 showed little effect of salinity on germination and up to 75% germination was observed at 0.75% salinity. EC 508311 was among highly susceptible genotypes, which showed no germination at 0.75% and only 15% germination at 0.25% salinity. EC 4017103, EC 401711, ISH 26/50/7, Multi-98-45, Raj 49/50 also belonged to susceptible group showing drastic reduction in germination even at low salinity stress. Genotypes like EC 400977, ISH 34/49, ISH 34/41, Penta 99-1, Raj Bundi, ES 99, ISH 32/8/1, Wardan S2, T 44- 4, T 45-1, ISH 8020B, ISH 34/8B, ISH 34/8Y, T 5-90-I, ISH 8020Y, ISH 5050 B, T 9-90-FM, showed moderate tolerance and gradual reduction in germination was observed with increasing salinity levels. Genotypes like ISH 32/34/1, ISH 34/5/1, T 5-90I-1 and ISH 5050Y showed better tolerance (little reduction in germination) at low salinity levels but drastic reduction at high salinity was observed. Bayuelo-Jimenez et al. (2002) observed that salinity stress delayed germination in all accession to varying degrees and exhibited high genetic potential within *Phaseolus* for salinity tolerance during germination. In *Sorghum bicolor* cultivars decrease in seed germination and shoot/root extension in response to NaCl salinity was largely attributed to ionic toxicity rather than to osmotic factors (Macharia et al., 1995). However, Mano et al. (1996)

suggested that effect of salt-stress on germination was mainly due to osmotic stress. Though tolerance at one developmental stage is unreliable for predicting the tolerance at other stages of development. Bernstein and Hayward (1958) observed that the first exposure of a crop to salinity stress occurs at the germinating stage and is likely to proceed further under higher surface soil salinity than would be the case for later growth stages. Hence, improving the uniformity and rapidity of seed germination under salinity might contribute significantly to the efficiency of stand establishment.

A.2 Survival

Almost all the plants survived up to 45 days at 0.25% salinity; however, high mortality was observed at higher salinity levels. In genotypes EC 329299, EC 400977, EC 400976, ISH 34/49, ISH 26/50/7, Multi-98-45, ISH 34/5/1, ISH 8020Y, 10 to 15% mortality was observed at 0.50% salinity whereas 50 to 100% of the seedlings perished at 0.75%. Genotype EC 318954 showed no mortality at 0.25% and low mortality (10 and 20%) at 0.50% and 0.75% salinity respectively. Genotypes Wardan, ISH 32/8/1, Wardan S2, ISH 32/34/1, T 44-4, T 5-90I-1, ISH 5050B, ISH 34/8B had moderate to high increase in mortality from 0.25 to 0.75% salinity. Genotypes EC 407709, ISH 34/41, ISH 34/11, T 45-1, ISH 8020B, ISH 34/8Y, T 5-90-I, T 9-90-FM were tolerant to prolonged salinity and less than 30% mortality was observed at 0.75% salinity. Among the highly susceptible group of genotypes EC 4017103, ES 99, Raj 49/50 high degree of mortality at lower salinity and near 100% mortality at 0.75% was observed. Low mortality at lower salinity (0.25%) and near 100% mortality at 0.50% and 0.75% salinity was observed among genotypes EC 508311, EC 401711, Penta 99, Raj Bundi, Penta 99-1, and ISH 5050Y.

A.3 Seedling vigour and growth

Early seedling vigour is an important selection criteria for salinity tolerance. Tolerance observed at germination, early seedling and the vegetative growth stage is of great importance because salinity tolerance at every stage of growth is of considerable value in determining the ultimate tolerance of the species (Shannon, 1984). Hence, in the present study seedling vigour in terms of plant height, root length, number of leaves and biomass on 20th day after inoculation of seeds was recorded. Growth of plants in saline conditions was found to be highly variable in genotypes showing their heterogeneous nature. The seedlings with reduced or no root growth were considered to be susceptible whereas the seedlings with normal root growth were considered to be tolerant as the development of

roots is of primary importance in the stand establishment of any crop/plant. Seedling data in each replication was recorded for susceptible/tolerant type of plants separately. Further, in order to see the survival and growth of plants after prolonged exposure to salinity, growth parameters of plants were recorded on 45th of seedling growth. Hereunder is discussed in brief the response of each genotype individually.

EC 329299: At 0.25% salinity overall growth response of this genotype was tolerant. Biomass production increased marginally as compared to control, growth of roots and shoot was good and the seedlings reached 3 leaf stage. The growth of seedlings at 0.50% was also satisfactory. Majority of the seedlings showed positive pattern of growth of roots and shoots. However, growth of seedlings at 0.75% was retarded. Few of the seedlings remained at cotyledonary leaf stage. Positive pattern of root and shoot growth with 1-2 leaves was observed in 37.5% of the seedlings. On 45th day of growth the average shoot length of the seedlings was reduced to about 50% at 0.25% salinity, with further decrease at higher salinity levels. The average biomass production under control condition was 418 mg, which reduced to 191, 121 and 50 mg at 0.25%, 0.50% and 0.75% salinity.

EC 318954: The genotype exhibited tolerant response upto 0.50% salinity. The seedlings had well developed roots, satisfactory height and 2-3 leaves at 0.25% salinity showing highly resistant behaviour. The growth of seedlings at 0.50% was also good. Majority of the seedlings showed positive growth pattern though growth of roots was not as vigorous as in 0.25% salinity but some plants had elongated and elaborate root system. The seedlings were mostly at 2-3 leaves stage. Only 11.4% of the seedlings exhibited susceptibility behavior to salinity. At 0.75% salinity growth of the seedlings was reduced. Many of the seedlings remained at cotyledonary leaf stage with inhibited development of roots. However, satisfactory growth of root, shoot and presence 2 leaves each was observed in 52% of the seedlings. On 45th day of growth the average shoot and root length, number of leaves and biomass of the seedlings was reduced marginally at lower salinity but drastic decrease was observed at higher salinity.

Wardan: Response of this genotype to varying levels of salinity was of susceptible nature. The growth of seedlings at 0.25% was slow with reduced shoot and root growth. Susceptible growth pattern was observed in 25% of the germinated seedlings with no roots or aerial, hair like roots and even the cotyledonary leaves did not emerge. Most of the seedlings at 0.50% remained in cotyledonary leaf stage. At 0.75% salinity even the cotyledonary leaves failed to emerge with no root development in most of the seedlings.

On 45^{th} day of *in vitro* growth, the root length, shoot length, biomass and number leaves were drastically reduced as compared to control.

EC 407709: The response of this genotype to different levels of salinity was found to be tolerant. The plants at 0.25% were mostly at 2-3 leaves stage. The growth of seedlings was good at 0.50% also. The height of few plants was even more than the plants at 0.25% salinity. Development of roots was good. The plants were at 2-3 leaves stage. Positive pattern of growth was observed in 24% of the seedlings at 0.75% salinity. The growth of these seedlings was at par to the resistant type of seedlings at 0.25% salinity. Thus, higher salinity level had little deleterious effect on the growth of these seedlings. However, remaining seedlings showed reduced growth, with single leaf or emerging cotyledonary leaves and aerial hair like roots or no roots at all. The average biomass of the plants on 45^{th} day of growth under control condition was 203 mg which was reduced to 89, 82 and 99mg at 0.25%, 0.50% and 0.75% salinity.

EC 400976: The genotype showed partially tolerant response to different levels of salinity. The growth of seedlings was satisfactory at 0.25% with 46.6% of the seedlings attaining good height with 2-3 leaves each. The other seedlings were at cotyledonary leaf stage, height was reduced with abnormal development of roots or no roots at all. The growth of seedlings at 0.50% exhibited mixed pattern. Positive pattern of growth having 1-3 leaves, satisfactory development of roots and shoots was observed in 35% of the seedlings the remaining seedlings had very inhibited growth. The growth of seedlings was further inhibited at 0.75%, even the cotyledonary leaves failed to emerge properly, root development almost completely inhibited and the height of seedlings very much reduced. On 45^{th} day of growth the average root and shoot length of the plants reduced significantly at 0.25% and 0.50% salinity respectively with 2 to 3 leaf formations. Biomass also reduced to half at 0.25% and ¼ at 0.50%.

EC 508311: The response of this genotype to different levels of salinity was susceptible. The growth of seedlings at 0.25% was inhibited to large extent. Most of the seedlings showed negative pattern of growth with reduced height, development of roots highly inhibited with mostly aerial, hair like roots or no roots. At 0.50% the growth of roots was completely inhibited and even the cotyledonary leaves failed to emerge. At 0.75% salinity, no germination was observed. On 45^{th} day of growth no plants survived at the higher salinity levels. Shoot length and biomass reduced to one third at 0.25% salinity along with drastic reduction in root length and number of leaves.

EC 4017103: The genotype exhibited highly susceptible response to different levels of salinity. At 0.25% salinity seedlings growth was inhibited with reduced height and poor development of roots. At 0.50% salinity, the growth and development of seedlings was almost completely inhibited with no development of roots and even the cotyledonary leaves failed to emerge. At 0.75% only one seed germinated. On 45^{th} day of growth root length was highly inhibited at 0.25% and 0.50% salinity levels and biomass drastically reduced.

EC 400977: Response of the genotype to different levels of salinity was of susceptible nature. At 0.25% salinity growth of seedlings was inhibited with negative geotropic growth of roots, reduced height having single leaf. The growth of seedlings at 0.50% was further reduced with almost complete inhibition of root growth. The plants were mostly at cotyledonary leaf stage. At 0.75% salinity the growth of seedlings was inhibited with absence of roots and even the cotyledonary leaves failed to emerge, except in one seedlings having 1 leaf. Most of the plants degenerated by 45^{th} day.

EC 401711: The overall response of this genotype was susceptible. At 0.25% salinity, 45% of the germinated seedlings showed positive pattern of growth with satisfactory height, 1-2 leaves and satisfactory root development while the other seedlings had reduced and abnormal pattern of growth of roots and only the cotyledonary leaves emerged. At 0.50% the growth of seedlings was poor; the seedlings were mostly at cotyledonary leaf stage, with few seedlings having single leaf and reduced height. Root development was highly inhibited. The growth of seedlings was highly inhibited at 0.75% salinity and even the cotyledonary leaves failed to emerge. On 45^{th} day of growth drastic reduction in shoot length, root length and biomass of the plants growing under stress condition was observed.

ISH 34/49: The response of this genotype was tolerant to low salinity levels. At 0.25% salinity growth of seedlings was good with fine root, shoot development and 2-3 leaves. Negative pattern of growth with reduced height and no roots was observed only in 6% of the total germinated seedlings. At 0.50% salinity, 29% of the seedlings had positive pattern of growth with satisfactory development of root and shoot. At 0.75% growth of seedlings was poor, with abnormal aerial hair like, or with no roots and 1 leaf. Positive pattern of growth with satisfactory growth of root, shoot and two leaves each was observed in 10% of the seedlings only. On 45^{th} day although shoot length, number of

leaves and weight of plant of the plants decreased at 0.25% and 0.50% salinity but the percent reduction was less as compared to that in root length.

<u>ISH 34/41</u>: The response of this genotype was susceptible. The shoots developed at 0.25% salinity but root development was almost inhibited. The seedlings had good height having 2-3 leaves. Similar growth pattern with increased negative effect of salinity was observed at 0.50% and 0.75% salinity. On 45^{th} day of growth shoot length reduced to nearly half even at 0.25% salinity additionally drastic loss in root length was noticed. However, leaf initiation was less affected indicating reduced internodal length. Biomass production was less affected at 0.25% and 0.50% but no plant survived at 0.75% salinity.

<u>ISH 34/11</u>: The genotype showed partial tolerant response to low salinity. At 0.25% salinity, 30% of the seedlings had positive pattern of growth and height of these seedlings was satisfactory with 1-2 leaves with satisfactory growth of roots. At 0.50% salinity, 20% of the seedlings had positive pattern of growth with proper development of roots and shoots. At 0.75% salinity, the growth of seedlings was retarded with mostly single leaf. Development of root was almost inhibited. On 45^{th} day of growth more decrease in shoot length was observed as compared to root. Number of leaves was less affected and biomass reduced to half at 0.25% followed with gradual decrease.

<u>Penta 99</u>: The genotype was tolerant up to 0.50% salinity. At 0.25% growth of majority of the seedlings was good with proper root development. Susceptible pattern of growth was observed in 26% of the seedlings with reduced height and abnormal growth of roots i.e. aerial/ hair like roots and 1-2 leaves. At 0.50% the growth of seedlings was retarded however, 28% of the seedlings exhibited positive pattern of growth with satisfactory shoot and root development. The susceptible ones had reduced height, abnormal growth of roots and mostly single leaf. At 0.75% salinity, growth of seedlings was retarded, development of roots was inhibited and the seedlings were mostly at cotyledonary leaf stage. Drastic reduction of growth at 0.75% is an indication that this genotype had the potential to grow at limited stress conditions. On 45^{th} day of growth gradual decrease in shoot length, root length, number of leaves and biomass at 0.25 and 0.50% salinity was observed.

<u>Raj Bundi</u>: The genotype was found to possess tolerance to limited salinity conditions. At 0.25% salinity majority of the seedlings had good height with two leaves each and positive root development. Negative pattern of growth with aerial/ hair like roots, reduced

height of plants and 1-2 leaves was observed in 26% of the total seedlings. However, at 0.50% salinity only 26% of the seedlings had positive pattern of growth and these plants had mostly single leaf. The other seedlings exhibited negative pattern of growth with aerial/hair like roots or no roots at all and reduced height. At 0.75% salinity the growth of seedlings was highly retarded, the seedlings had reduced height, with only the cotyledonary leaves emerging. Decrease in shoot length and root length but less degree of reduction in number of leaves and biomass at 0.25 and 0.50% salinity was observed by 45 days of growth.

Penta 99-1: The genotype had certain degree of tolerance to low salinity conditions only. The growth of seedlings was slightly inhibited at 0.25% salinity and 46% of the seedlings had positive pattern of growth with satisfactory height, fine development of roots and 2-3 leaves. At 0.50% salinity the growth of seedlings was retarded with most of the seedlings having susceptible pattern of growth having aerial/hair like roots or no roots. Resistant pattern of growth was observed only in 14% of the total seedlings, though the height of these seedlings was reduced with 1 leaf each. At 0.75% the growth of seedlings was very much retarded with 1-2 leaves and aerial/ hair like roots. On 45th day of growth plants surviving at 0.25% salinity only showed reduced shoot length, root length, number of leaves and biomass.

ES 99: This genotype had some degree of tolerance to low and medium salinity. At 0.25% salinity, 27% of the seedlings had positive pattern of growth with satisfactory height, 1-2 leaves and roots. The growth of seedlings at 0.50% salinity was reduced with majority of seedlings having susceptible pattern of growth. Positive pattern of growth with satisfactory height, fine growth of roots and 1-leaf each was observed in 27% of the seedlings. The growth of seedlings at 0.75% salinity was almost inhibited. Even the cotyledonary leaves failed to emerge properly. The development of roots was almost inhibited. On 45th day of growth plants survived only at 0.25% salinity and reduced shoot length, root length, number of leaves and biomass was observed.

ISH 32/8/1: The response of this genotype to different salinity levels was susceptible. Even at 0.25% salinity growth of seedlings was inhibited; the height of seedlings reduced and most of the plants were at cotyledonary stage, the development of root was highly inhibited. The growth of seedlings at 0.50% salinity was stunted with most of the seedlings at cotyledonary leaf stage. At 0.75% salinity only 20% of the seeds germinated. The growth of seedlings was highly inhibited and even the cotyledonary leaves failed to

emerge properly. Roots also failed to develop. Growth in saline conditions for 45 days showed less affect on shoot length as compared to root length. Number of leaves was adversely affected and biomass also reduced to half both at 0.25 and 0.50% salinity.

Wardan S 2: The response of this genotype to different saline conditions was susceptible. At 0.25% salinity growth of seedlings was reduced, development of root inhibited, height of plants reduced and seedling mostly at 1 leaf stage. At 0.50% salinity growth of seedlings was reduced, most of the seedlings were at 1 leaf stage and without roots. Positive pattern of growth with proper development of both roots and shoots having 2 leaves was observed only in 5% of the seedlings. At 0.75% salinity only 28.3% seeds germinated. Growth of seedlings was highly reduced and even cotyledonary leaves failed to emerge and there was no root development. On 45th day of growth shoot length, root length and number of leaves were reduced but biomass at 0.25% was not much affected.

ISH 26/50/7: The growth pattern of this genotype was found to be susceptible to saline conditions. At 0.25% salinity most of the seedlings had susceptible pattern of growth with reduced height, abnormal development of root or no roots at all and with single leaf or with cotyledonary leaves. Only 28% seedlings had positive pattern of growth having good shoot growth, 2-3 leaves and satisfactory root development. Thus, the number of susceptible seedlings outnumbered the resistant seedlings. The growth of seedlings was retarded to great extent at 0.50% salinity and development of roots highly inhibited. Even the cotyledonary leaves failed to emerge properly. Few seeds that germinated at 0.75% showed retarded growth, with no development of roots and leaves. On 45th day of growth shoot length, root length and number of leaves were drastically reduced but biomass at 0.25% was not much affected, even at 0.50% salinity yield reduction was 50% although at 0.75% there was drastic reduction in yield.

ISH 32/34/1: This genotype showed some tolerance at low salinity. At 0.25% salinity the growth of seedlings showed mixed pattern. Nearly 1/3 seedlings had positive pattern of growth, satisfactory height, good roots development and two leaves. At 0.50%, the plants had reduced height with most of the seedlings at cotyledonary leaf stage and inhibited root development. At 0.75% salinity seedling growth was retarded with no roots and leaf development. Some of the seedlings started to degenerate by 20th day. On 45th day of growth shoot length, root length and number of leaves at 0.25% showed minimum reduction but at 0.50% and 0.75% salinity growth of the plants was drastically reduced.

The average biomass marginally increased at 0.25% whereas at 0.50% and 0.75% it decreased.

__Multi-98-45__: The growth response of this genotype to saline conditions was susceptible. At 0.25% salinity the growth of seedlings was reduced with only 26% of the seedlings having good growth, 1-2 leaves and the development of roots satisfactory. At 0.50% salinity the growth of seedlings was highly retarded and even the cotyledonary leaves failed to emerge. At 0.75% salinity only two seeds germinated by 20th day. The growth of these seedlings was highly retarded. On 45th day of growth shoot length, root length and number of leaves showed gradual decrease at 0.25% and 0.50% salinity. Biomass was reduced approx 40% at 0.25% and 50% at 0.50% salinity.

ISH 34/5/1: The growth of seedlings was inhibited under saline conditions with only 16% of the seedlings having satisfactory and positive pattern of growth at 0.25% salinity. The other seedlings had negative pattern of growth with aerial/hair like root or no development of roots; reduced height with 1-2 leaves and few with cotyledonary leaves only. At 0.50% salinity the development of root was highly inhibited with most of the seedlings possessing no roots or aerial/hair like roots. Positive pattern of growth was observed only in 16% of the total seedlings. At 0.75% salinity development of root was totally inhibited and cotyledonary leaves failed to emerge. On 45th day of growth drastic reduction in shoot and root length was observed but number of leaves and biomass were not much affected.

Raj 49/50: The response of this genotype was highly susceptible to salinity. No germination was observed at salinity level 0.50% and 0.75% showing deleterious effect of salinity. Few seed that germinated at 0.25% salinity level had very poor growth. Growth of radicle and plumule were highly inhibited and even the cotyledonary leaves sometimes failed to emerge. On 45th day of growth no plants survived at any salinity.

T 5-90I-1: The response of this genotype was found to be susceptible to saline conditions. At 0.25% salinity the growth of seedlings was retarded. Most of the seedlings had reduced height, abnormal or no growth of roots and 1-2 leaves. Only 21% of the total seedlings had positive pattern of growth. At 0.50%, the growth of seedlings was highly retarded. The seedlings had reduced height with only cotyledonary leaves and abnormal or no roots. At 0.75% majority of the seedlings had susceptible pattern of growth with abnormal development of roots i.e. aerial/ hair-like roots. On 45th day of growth gradual

reduction in shoot length, root length and number of leaves was observed however, biomass increased at 0.25% salinity but decreased with increasing salinity.

<u>T 45-1</u>: The response of this genotype was partially tolerant. At 0.25% salinity the growth of seedlings was satisfactory with 44% of the seedlings having positive pattern of growth with 2-3 leaves. The growth of seedlings at 0.50% salinity was reduced, most of the seedlings had hanging aerial roots and seedlings were mostly at cotyledonary leaf stage with some seedlings having 2 leaves. One plant having no roots at all had extensive growths of shoot, the leaves were much larger compared to other seedlings leaves. Positive pattern of growth of root and shoot was observed in 23% of the seedlings. The height of these seedlings was satisfactory with 1-2 leaves and positive development of roots. Most of the seedlings at 0.75% salinity had susceptible pattern of growth with no root and single leaf or only the cotyledonary leaves. On 45th day of growth drastic and gradual reduction in shoot length, root length, number of leaves and biomass observed.

<u>T 44 - 4</u>: Growth response of this genotype was tolerant to low saline conditions only. At 0.25% salinity most of the seedlings had positive pattern of growth, the height of such seedlings was satisfactory with 2-3 leaves and fine development of roots. The seedlings having susceptible pattern of growth were mostly having 1 leaf each or only cotyledonary leaves. The height of such seedlings was highly reduced with abnormal growth of roots. At 0.50% salinity the growth of majority of the seedlings was highly retarded with reduced height, development of root either absent or with aerial/hair like roots and no leaf development. At 0.75% salinity the growth of most of the seedlings was highly inhibited. Even the cotyledonary leaves failed to emerge. On 45th day of growth reduction in shoot length, root length, number of leaves and biomass observed.

<u>ISH 8020B</u>: The response of this genotype was tolerant to saline conditions. The growth of majority of the seedlings at 0.25% was good and positive. At 0.50% salinity the seedlings showed mixed pattern of growth with majority of the seedlings having susceptible type of growth. Positive pattern of growth was observed in 22% of the seedlings present. At 0.75% salinity also seedlings showed mixed pattern of growth with majority of the seedlings having susceptible pattern of growth, however, 33% of the seedlings had positive growth with 2-3 leaves and fine development of roots. On 45th day of growth reduction in shoot length, root length, number of leaves and biomass was observed at all salinity levels.

ISH 8020 Y: The response of this genotype was susceptible to saline conditions. At 0.25% salinity the growth was of mixed type with some seedlings having susceptible type of growth and some resistant type of growth. At 0.50% salinity the growth of seedlings was inhibited with majority of the seedlings having susceptible pattern of growth, only 11% of the seedlings had positive pattern of growth. At 0.75% salinity the growth of seedlings was highly inhibited. The seedlings had very poor growth of shoot with reduced height and total absence of any type of roots. On 45th day of growth reduction in shoot length, root length, number of leaves and biomass was observed.

ISH 5050 B: The response of this genotype was tolerant to low salinity. At 0.25% the growth of the seedlings had a mixed pattern. The height of the susceptible type of seedlings was reduced compared to the resistant type. The growth of roots was highly inhibited in the susceptible seedlings and roots were aerial and hair–like. At 0.50% salinity the growth of shoot in majority of the seedlings was identical, but the susceptible type of seedlings had abnormal root growth with aerial hair- like roots or no development of roots at all. At 0.75% salinity development of root was almost inhibited. Most of the seedlings were at cotyledonary leaf stage. On 45th day of growth moderate and gradual reduction in shoot length, root length, number of leaves and biomass was observed.

ISH 5050Y: The genotype possessed limited tolerance to low salinity. At 0.25% salinity the seedlings had mixed pattern of growth with some having susceptible and some resistant type of growth. At 0.50% salinity the majority of the seedlings had susceptible pattern of growth with abnormal development of roots or no roots at all, although the growth of shoot in these seedlings was satisfactory with 1-2 leaves. Resistant type of growth with fine and positive development of roots and shoot with 1-2 leaves was observed only in 13% of the seedlings. At 0.75% salinity the growth of seedlings was highly inhibited. On 45th day of growth plants survived only at 0.25% salinity showed reduction in shoot length, root length, number of leaves and biomass.

ISH 34/8B: The growth response was tolerant to low levels of salinity. The growth of some seedlings was susceptible whereas growth of other seedlings was resistant type. The average biomass of the susceptible type of seedlings at 0.25% and 0.50% salinity was more than at control due to the thick and enlarged leaves whereas in the resistant type of seedlings biomass production decreased gradually with increasing salinity level. Growth of the seedlings at 0.50% was reduced and only cotyledonary leaves emerged and almost total inhibition of root development. Positive pattern of growth was observed only in 28%

of the seedlings. The growth of seedlings at 0.75% was highly inhibited with majority of the seedlings having susceptible pattern of growth. On 45th day of growth moderate and gradual reduction in shoot length, root length, number of leaves and biomass was observed.

ISH 34/8Y: The growth response indicated tolerance to low levels of salinity. Good number of seedlings at 0.25% had positive growth pattern and the seedlings reached 3-4 leaf stages with fine development of roots. Majority of the seedlings at 0.50% had susceptible growth pattern with reduced height of the seedlings, abnormal development of roots and seedlings at cotyledonary leaf stage. The growth and development of seedlings at 0.75% was inhibited. Most of the seedlings had reduced height with retarded and abnormal growth of roots. On 45th day of growth moderate and gradual reduction in shoot length, root length, number of leaves and biomass was observed.

T 5-90-I: The growth response was tolerant to low levels of salinity. The growth of majority of the seedlings was positive at 0.25% salinity and reached 3-4 leaf stages with fine development of roots. Majority of the seedlings at 0.50% salinity exhibited susceptible pattern of growth with reduced height of the seedlings and the seedlings mostly at cotyledonary leaf stage. The growth of seedlings at 0.75% salinity was highly retarded. The height of seedlings significantly reduced with abnormal growth of roots or no development of any roots at all. On 45th day of growth moderate and gradual reduction in shoot length, root length and number of leaves observed, however, biomass increased at 0.25% as well as at 0.50% salinity.

T 9-90-FM: The genotype was found to possess tolerance to low levels of salinity. At 0.25% salinity the growth of seedlings was satisfactory, the seedlings attainted good height, the development of roots positive and the seedlings at 2-3 leaf stage. At 0.50% salinity the seedlings had reduced height, the development of roots inhibited, majority of the plants with hair-like aerial roots, and mostly at cotyledonary leaf stage or 1- leaf stage. The leaves were thickened and enlarged. At 0.75% salinity the growth of seedlings was highly inhibited, development of roots inhibited and the plants were mostly at cotyledonary leaf stage. On 45th day of growth moderate and gradual reduction in shoot length, root length, number of leaves and biomass was observed.

A.4. Analysis of variance

Single factor analysis of variance for germination, shoot length, root length, number of leaves and plant weight of 20 days old seedling revealed significant differences for all the

traits under study under salinity treatments among all the genotypes. However, genotypes EC 407709, ISH 34/49, ISH 32/8/1 and ISH 8020B showed non- significant variation for number of leaves. Similarly genotypes EC 318954, EC 4017103, ISH 34/49, ISH 34/11, Penta 99, Raj Bundi and Penta 99-1 showed non- significant variation for plant weight.

Two factor analysis of variance for germination, shoot length, root length, number of leaves and plant weight revealed significant differences both at genotypic and salinity levels for all the traits under study. Indicating thereby that both genotypes and different levels of salinity treatments had significant affect on the parameters observed

A.5. Susceptibility Index

Based on relative performance of genotypes at 0.75% salinity vis-à-vis normal condition susceptibility index was calculated for various traits under observation (Table 35-.). Higher values of susceptibility index are treated as susceptible and lower values as indicative of tolerance (Bayuelo- Jimenez et al., 2002).

Germination: The sensitivity index for germination of seeds was highest for the genotypes EC 508311 (1.39), Raj 49/50 (1.39), ISH 32/34/1 (1.27), T 5-90I-1 (1.23), and E C 401711 (1.23), indicating their susceptibility to high levels of salinity whereas the genotypes EC 407709 (0.29), ISH 34/11 (0.32), Penta 99 (0.52), Raj Bundi (0.71) and ISH 34/41 (0.72) recorded low sensitivity indices showing their tolerant nature

Shoot length: Sensitivity index for shoot length was highest among genotypes Raj 49/50, EC 508311, ISH 32/34/1 and ISH 26/50/7, thus showing susceptible nature. Genotypes EC 407709 (0.19), ISH 34/49 (0.35), Raj Bundi (0.80), ISH 34/11 (0.77) and Penta 99 (0.82) showed tolerance towards salinity as indicated by low SSI values. Thus, it appears genotypes EC 407709, ISH 34/11 and Penta 99 possessed some mechanism for salt tolerance as far as shoot growth was concerned. The genotype EC 407709 had the maximum growth in both susceptible (2.58 cm) and resistant category of plants (10.72 cm). Thus, productivity criteria of selection under stress conditions place this genotype to be most tolerant. Shoot length is reported found to significantly decrease with increasing NaCl concentration in Lucerne (Al-Khatib et al., 1994). They advocated selection between and within cultivars might lead to increased salt tolerance in this species. In the present study also shoot length was reduced and differential response of seedlings in same genotypes was observed indicating chances for intragenotypic selection.

Root length: Sensitivity index for root growth was highest in the genotypes EC 508311 (1.20), Raj 49/50 (1.20), ISH 5050B (1.14), ISH 8020Y (1.14) and T 44-4 (1.12) whereas

the genotypes EC 407709 (0.33), ISH 34/49 (0.66), EC 400977 (0.75), Penta 99 (0.82) and Raj Bundi (0.83) had minimum reduction in the root length as indicated by their low SSI values. Thus, based on growth and development of roots genotypes EC 508311 and Raj 49/50 were highly sensitive whereas genotypes EC 407709, EC 400977, ISH 34/49, Penta 99 and Raj Bundi were tolerant. Genotype EC 407709 had maximum root growth in both the susceptible (2.81 cm) and resistant category of plants (3.95 cm).

Number of leaves: The sensitivity index for number of leaves in the genotypes EC 508311 (1.77), Raj 49/50 (1.77), ISH 5050Y (1.35), ISH 34/8B (1.32) and T 9-90-FM (1.30) were found to be high. The genotypes EC 407709 (0.06), ISH 34/49 (0.10), ISH 32/8/1 (0.18), EC 400977 (0.41) and EC 401711 (0.42) were least sensitive for leaf growth as indicated by their low SSI values. Of these genotypes EC 400977, EC 401711 and ISH 32/8/1 showed low SSI values in susceptible group of plants also indicating that these genotypes possess lesser intra-genotypic variation. Genotype EC 407709 was least sensitive based on data recorded on resistant category of plants while it was susceptible based on data of susceptible group of plants indicating higher intragenotypic variation for salinity tolerance. Considerable intra-ecotype variation for salinity and alkalinity tolerance in *Trifolium alexandrinum* L has been reported earlier also (Chandramohan, 2001). This indicates that intragenotypic variation exists in *T. alexandrinum* which can be exploited in selection for salinity tolerant plants. Intra and inter genotypic variation for salt tolerance has also been reported in Lucerne by Al- Khatib et al. (1994). Intra-cultivar variations have been identified in Lucerne and *T. repens* allowing selection programme to be undertaken (Rogers and Noble, 1990). Both these species are predominantly out crossed and posses large intra-cultivar genetic variation.

Weight of plant: The sensitivity index was highest in the genotypes EC 508311 (2.68), Raj 49/50 (2.68), ISH 34/8B (2.25), T 44-4 (2.19) and Multi-98-45 (2.17), thus indicating their susceptibility to high levels of salinity, whereas the genotypes Penta 99-1 (–16.09), T 9-90-FM (–3.72), Raj Bundi (0.13), ISH 34/49 (0.19) and Penta 99 (0.28) were found to be least sensitive. Genotypes Penta 99-1 isolation and T 9-90-FM even showed higher biomass at 0.75% salinity than control condition. Thus, the genotypes EC 508311, Raj 49/50, and T 44-4 were sensitive to salinity whereas Penta 99-1, Penta 99, Raj Bundi and ISH 34/49 were least sensitive to high salinity levels for biomass production. The genotypes with minimum SSI values can be used to identify the exact mechanism involved in adapting to salinity as well as identifying genes for salt tolerance.

Thus, the present study resulted in identifying different genotypes which showed susceptible, moderately tolerant and highly tolerant nature of the genotypes as indicated by germination, shoot/root growth and total biomass production. For example EC 407709, ISH 34/11, Penta 99, Raj Bundi and ISH 34/41 were tolerant for germination but Penta 99-1, T 9-90FM, Raj Bundi, ISH 34/49 and Penta 99 were tolerant for biomass production in terms of reduction as compared to their respective control. However, based on total biomass production genotypes T 9-90FM, EC 407709, EC 329299, EC 318954 and T 5-90-I emerged most tolerant. Similarly the genotypes EC 407709, EC 318954, EC 329299 and ISH 8020B were found to tolerant for growth of roots which can be regarded as the one of the most important criteria in selection for salt tolerance indicating that genes for tolerance at various growth stages are scattered across genotypes and can be used in pyramiding of genes for developing a salt tolerant variety/cultivar. Egyptian clover, although considered to be 95% self incompatible in Egypt, is represented by range of population with variable degree of self fertility (Roy et al., 2005). Hence, possibility of existence of intra-genotypic variation for response to salinity is expected. The study clearly established high degree of intragenotypic variation for response to salinity in some genotypes whereas some genotypes exhibited negligible intragenotypic variation which can be linked to their genetic makeup. Some genotypes in the study may in fact be a heterogeneous population because of their high out crossing nature whereas other genotypes may be genetically highly homogeneous due to their self fertile nature. Hence, identification of tolerant lines is important.

Foolad and Jones (1993) advocated that salt tolerance at germination and at the seedling stage to be controlled by more than one gene and are highly influenced by salt concentrations, however, salinity under field conditions is complicated by the heterogeneity in salt concentrations at different depths in the soil, time and space. Hence, evaluation of germplasm should be done after providing best possible uniform condition.

Survival of plants for 45 days in *in vitro* condition as well as in sand culture condition at different salinity levels from 0.25% to 1% salinity confirm the salt tolerant nature of the crop. Earlier reports also confirm that the crop possesses tolerance for salinity (Raheja, 1966; Winter and Lauchli, 1982). The species is also reported to possess better salt tolerance than *T. pratens* tested at 50, 100, 150 and 200 mM NaCl salinity levels (Winter and Lauchli, 1982).

Development of broad based germplasm pools by intercrossing salt tolerant lines of diverse origin followed by mass propagation under highly saline conditions can be a

Table 35. Saline Susceptibility Index (SSI) analysis in 20 day old seedlings.									
Genotypes	% germination	Shoot length		Root length		No of leaves		Weight of plant	
		S	R	S	R	S	R	S	R
EC 329299	1.00	1.12	1.11	1.14	1.02	1.15	1.18	1.25	1.96
EC 318954	0.84	1.11	1.07	1.18	1.07	1.22	1.18	1.25	0.38
Wardan	1.05	0.99	1.04	1.08	1.10	0.82	0.97	0.95	1.71
EC 407709	0.29	0.99	0.19	0.49	0.33	1.07	0.06	1.01	0.33
EC400976	1.19	1.03	1.12	0.87	0.94	1.07	1.16	1.17	1.89
EC 508311	1.39	1.30	1.37	1.20	1.20	1.64	1.77	1.61	2.68
EC4017103	1.19	0.99	1.02	0.91	0.92	0.62	0.68	1.49	2.01
EC400977	1.14	0.85	0.90	0.69	0.75	0.29	0.41	1.01	1.49
EC401711	1.23	1.14	1.20	0.99	1.01	0.38	0.42	1.23	2.05
ISH 34/49	0.90	0.83	0.35	0.81	0.66	0.83	0.10	0.32	0.19
ISH 34/41	0.72	0.99	1.07	1.03	0.98	0.99	1.11	0.73	1.49
ISH 34/11	0.32	0.71	0.77	0.89	0.91	0.90	1.03	0.27	0.49
Penta 99	0.76	0.76	0.82	0.73	0.82	0.57	0.67	0.09	0.28
Raj Bundi	0.71	0.72	0.80	0.80	0.83	0.67	0.80	-0.24	0.13
Penta 99-1	0.52	0.58	0.69	0.85	0.89	0.69	0.69	-1.76	-16.09
ES 99	1.03	0.86	0.93	0.97	0.97	0.86	0.89	1.00	1.58
ISH 32/8/1	1.06	0.71	0.76	0.97	1.02	0.16	0.18	0.80	1.43
Wardan S2	0.94	1.08	1.15	1.00	1.02	1.03	1.12	0.83	1.39
ISH 26/50/7	1.19	1.15	1.23	1.10	1.09	1.01	1.24	1.16	2.01
ISH 32/34/1	1.27	1.16	1.23	0.99	1.04	0.96	1.11	1.12	1.93
Multi 98-45	1.19	1.08	1.18	1.08	1.10	1.03	1.13	1.12	2.17
ISH 34/5/1	1.18	1.08	1.20	1.09	1.05	0.96	1.14	0.89	1.74
Raj 49/50	1.39	1.30	1.37	1.20	1.20	1.64	1.77	1.61	2.68
T 5-90l-1	1.23	0.66	0.84	0.97	1.10	0.94	1.12	0.84	1.83
T 45-1	1.18	0.94	1.11	0.97	1.04	0.96	1.20	0.64	1.61
T 44-4	1.12	0.96	1.09	1.05	1.12	1.12	1.16	1.27	2.19
ISH 8020B	1.04	1.11	0.98	1.11	1.08	1.14	0.91	1.07	1.90
ISH 8020Y	1.11	1.06	1.19	1.10	1.14	0.97	1.24	0.89	1.83
ISH 5050B	0.82	0.98	1.11	1.10	1.14	1.18	1.35	0.50	1.51
ISH 5050Y	1.26	0.97	1.11	1.08	1.11	0.99	1.24	0.80	1.72
ISH 34/8B	0.96	1.06	1.22	0.98	1.10	1.07	1.32	1.05	2.25
ISH 34/8Y	0.93	0.93	0.97	1.06	0.92	1.20	1.25	1.00	1.38
T 5-90-I	1.11	1.07	1.16	1.07	1.06	1.17	1.18	1.28	1.92
T-9-90FM	1.15	1.09	1.20	1.09	1.10	1.15	1.30	1.13	-3.73

realistic approach. These enriched and dynamic populations would then form the basic materials in breeding for salt tolerance (Jana, 1993). The percent reduction in seedling vigour followed by percent reduction in seed germination has been advocated as better selection indices for salt tolerance (Thakral et al., 2001).

B. Biochemical studies

B. 1. Isozymic analysis

Isozymic banding pattern of SOD (Super Oxide Dismutase), PRX (Peroxidase) and Est. (Esterase) under 3 levels of salinity (0.25%, 0.50% and 0.75%) in 20 day old seedlings was compared with seedlings growing under control condition. The study showed presence of 4 SOD, 11 PRX and 18 Esterase bands.

SOD banding pattern revealed polymorphism for band no. 4 only. The band was observed in 0.25% salinity treated plants of EC 4017103, ISH 32/34/1 and 0.50% and 0.75% treated plants in EC 400977 and ISH 34/8B. Additionally this band was present only in the control plants of genotypes Wardan S2 and ISH 34/8Y. The frequency of band 1, 2, 3, and 4 under control condition was 92, 92, 92 and 60% which increased among resistant plants at 0.25% salinity but in the susceptible plants it was almost similar to control, band 4 in resistant plants growing at 0.75% salinity had 100% band frequency whereas in the susceptible plants the frequency was less compared to control indicating that increased SOD activity provided some tolerance mechanism among resistant plants at low salinity levels. The plants having susceptible nature did not exhibit increased frequency of SOD bands. This was evidenced with the fact that frequency of band at 0.50% and control was almost equal

Peroxidase band 3, 5 and 8 were present in the susceptible plants only under different levels of salt stress among the genotypes EC 4017103, EC 400977, EC 401711, ISH 34/11, ES 99, Wardan S2 and EC 407709. However, in EC 329299 and EC 400976 band 3 and 8 were present in both susceptible and resistant plants under salt stress. In the genotypes EC 508311, ISH 32/34/1 and Multi 98-45 band 8, 10 and 11 were present in stressed plants only. In ISH 5050B band 8 was present only in the resistant plants at 0.25% salinity whereas in ISH 8020Y this band was present in both susceptible and resistant plants as well as under control condition. The frequency of band 2 in the control plants was 32% which reduced in the resistant plants growing at different salinity levels. Contrary to this, band 3 and 8 had substantial increased band frequency in plants growing

under stress. Similarly band 10 and 11 also exhibited increased frequency at 0.50% and 0.75% salinity. The intensity of bands indicates the relative quantity of isozyme produced. As regards intensity of peroxidase bands in control plants vis-à-vis stressed plants, no clear trend for increase or decrease in intensity could be established. However, sporadic changes in the increased intensity of band 1 in genotypes Wardan S2, ISH 26/50/7, EC 407709 and Multi 98-45 and band 2 in the genotypes Wardan S2 and Multi 98-45 was observed.

Esterase bands 6, 8 and 9 were observed alone or in combination in the plants growing in saline condition in genotypes like EC 4017103, EC 400977, EC 401711, EC 329299, EC 318954, EC 400976, ISH 34/5/1, T 45-1, EC 407709, ISH 5050B and ISH 8020Y. Band 8 was also present in control plants of T 45-1 and EC 407709. Band 13 in EC 400977, band 7 and 14 in ISH 34/41, band 5 in ISH 34/11, band 10, 14, 16 and 17 in Wardan S2, band 17 in EC 329299 and EC 318954, band 7 in Wardan, band 1, 5, 12 and 14 in EC 407709, band 18 and 11 in EC 400976, band 8, 14 and 15 in EC 508311, band 14 in Multi-98-45, band 16 in T 44-4, band 14 and 16 in ISH 5050B and band 16 in ISH 8020Y were seen only in plants growing in stress condition. EST band 10, 13, 14 and 16 alone or in combination had increased band intensity in the genotype EC 400976, ISH 34/5/1, Wardan and EC 329299. Thus, there were no specific bands associated with saline conditions across genotypes. However, band 6, 8 and 9 might have some significance as these bands appeared only in saline conditions in 11 genotypes. Dionisiosese and Tobita (1998) also reported decline in SOD activity and an increase in peroxidase activity in the salt sensitive rice varieties in response to salt-stress. In the genotype Wardan the band intensity at all salinity treatments was greater than at control. Intensity of bands reflects their quantitative expression. Such findings are also reported in other crops. For example, peroxidase isozymic band number 7 had darker intensity in rye grass at 0.3% salt concentration (He et al., 1992). The use of isozyme as markers has also been an approach of considerable value. An association of alleles of a peroxidase locus with salt-tolerance during germination in *Stylosanthes* has been reported (Lovato and Martins, 1997). Similarly, quantitative and qualitative variations in amylase composition in the calluses and esterase composition in the root tissue of seedlings under salt-stress was noticed among lines of rice, however, there was no appreciable isozymic variation in the leaf tissue.

B. 2. Native Protein

Selected eight genotypes representing 3 different ecotypes, tetraploid and interspecific hybrids were grown in pots at four salinity levels and native protein studies conducted on 25th day after sowing.

The study indicated appearance of native protein bands in response to saline conditions. However, the response was genotype specific with appearance of different mobility bands in different genotypes. The bands appearing only in saline conditions either alone or jointly were band 1, 2 and 3 (Wardan, EC 407709, T 45-1 and ISH 8020B), band 8, 10, 11 and 21 (EC 318954), band 3, 4, 7, 8, 9, 10, 11, 12 and 14 (EC 4017103), band 4, 9, 10, 11 and 15 (tetraploid genotype T 5-90I-1) and band 8 (EC 329299). Certain bands present in control plants were not observed in saline condition e.g. Band 6 and 18 in Wardan, band 11 in ISH 8020B and band 21 in EC 4017103. Certain bands appeared only in low salinity conditions. In tetraploid genotype T 45-1 band 6 and 13 were present only in the plants growing at 0.25% salinity. Similarly in genotype ISH 8020B band 17 was present only in the plants growing at 0.25% salinity and band 22 were present only at 0.25% and 0.50% salinity level in EC 329299.

As defense mechanism, plants synthesize certain proteins in higher quantity. In the present study, out of total 20 bands observed among different genotypes, band 2 in the genotype EC 329299, band 18 in the genotype EC 318954, band 19 in the genotype T 5-90I-1 and band 5 in the genotype EC 318954, EC 4017103, and T 5-90I-1 had greater intensity at all salinity treatments compared to control indicating their higher concentration in stressed plants.

Some proteins were synthesized in higher quantity only at higher salinity levels as expressed by their darker bands in the protein-banding pattern. For example band 4 in the genotype EC 318954 and T 5-90I-1 had increased intensity at higher salinity whereas in the genotype EC 329299 greater band intensity was observed at 0.25%, 0.50% and control conditions as compared to 0.75% salinity level. Band No.11 had increased band intensity at higher salinity level i.e. 0.75% and 1% whereas at 0.25% 0.50% and control condition it was reduced, in the genotype Wardan. Contrary to above situation it appeared that synthesis of certain proteins was masked in stressed condition that is why increased band intensity was observed in the control plants as compared to stressed plants for example band 14 and 16 in the genotypes T 45-1 and ISH 8020B had greater band intensity at 0.25% salinity but as the level of salinity increased the intensity decreased.

B. 3. SDS Protein

Selected eight genotypes representing 3 different ecotypes, tetraploid and interspecific hybrids were grown in pots at four salinity levels and SDS protein studies conducted on 25th day after sowing.

A total of 27 SDS polypeptide bands were identified among different genotypes. However, no definite pattern for appearance or disappearance of bands under saline condition as compared to control condition was observed. Most of the bands were common in stressed as well as non-stressed plants of the same genotype. Certain bands were seen at some salinity levels but absent in other salinity levels and control plants, thus indicating no definite pattern for stressed/non-stressed plants. Excluding the common bands following bands were unevenly present among different genotypes:

Wardan: Band 6 and 7 were absent in 0.25% salinity but present in all other salinity level and control plants. The band 13 was absent only at 1% salinity.

EC 407709: Band No. 6 and 7 present only at 0.25% salinity and under control condition.

T 45-1: Band 2 present at all salinity levels and band 4 present only at low salinity and control condition.

ISH 8020B: Band 6 was present at 0.25% salinity and control condition. The Band 9, 10, 13 and 14 absent only at 1% salinity level and band 19 present at 0.25%, 0.50% and control condition only.

EC 318954: Band 4 and 5 present only at 0.25%, 0.50% salinity and control plants. Band 7 and 20 present only at low salinity and control conditions.

EC 329299: Band 2, 3, 4, 5, 14, 16 and 21 absent only at 0.75% salinity. Band 7 present only at 0.25% salinity and control plants. Band 13 was present only under control plants and band 20 was present only at salinity 0.25% and 0.50%.

EC 4017103: Band 13 and 21 salt specific and present at all treatments.

T 5-90I-1: Band 2 present only at low salinity and control condition. Band 13, 19, 20 and 21 present at all salinity levels whereas band 16 present only under control plants.

B. 4. Sodium and Potassium ion estimation

Selected eight genotypes representing 3 different ecotypes, tetraploid and interspecific hybrids were grown in pots at four salinity levels and Sodium and Potassium ion estimation studies conducted on 25th day after sowing.

Na^+ concentration in roots of different genotypes increased with increasing salinity. In EC 329299 however, it was higher in control plants as compared to 0.25% and 0.75%.

Similarly in EC 318954 also it was almost at par with 0.25 and 0.75%.

In shoot portion the Na^+ concentration increased with increasing salinity in EC 329299, but it was still higher in control plants indicating its exclusion mechanism which restricted transport of Na^+ from roots to shoot portion. In EC 318954 also the Na^+ was more or less equal in control and the plants under stress condition. Variety Wardan also recorded decreased level of Na^+ concentration among plants growing in stress condition. Genotypes like EC 407709, T 45-1, T 5-90I-1 recorded increased level of Na^+ concentration in shoot in the plants growing under stress. Variation in salt tolerance and ion accumulation among subterranean clover cultivars was evaluated by Shannon and Noble (1995). They advocated that high productivity under saline stress was positively correlated with restricted Na^+ uptake in the shoot and the maintenance of high K^+ / Na^+ ratio.

K^+ in root increased with increasing salinity among three genotypes, remained more or less same among two genotypes whereas it decreased with increasing salinity among two genotypes. Decreasing K^+ level with increasing salinity can be considered as indication of resistant nature of the genotypes whereas more or less same level of K^+ in control and stressed plants is indicative of tolerance. As a whole EC 318954 showed minimum K^+ concentration in roots and EC 4017103 and T 5-90I-1 showed maximum K^+ concentration in the roots. Higher K^+ ion in control as compared to stressed plants also indicated that plants preferred to exclude K^+ uptake under stressed condition.

K^+ in shoot increased under stressed condition in two genotypes, remained more or less same among three genotypes whereas it decreased with increasing salinity among four genotypes. As a whole in the shoot also EC 318954 showed minimum K^+ concentration and EC 4017103 and T 5-90I-1 showed maximum K^+ concentration. Higher K^+ ion in control as compared to stressed plants also indicated that plants preferred to exclude K^+ uptake under stressed condition.

Under the experimental condition, the Saidi genotype EC 329299 showed better tolerance mechanism to increasing levels of NaCl treatment as the roots had high K^+ content over the whole salinity range (as compared with control). The root Na^+: K^+ ratio was also low or almost equal to control. This genotype had low content of Na^+ in the shoot region. Thus, low Na^+ in the shoot and high K^+ in the root may be regarded as the mechanism adopted to cope with salinity.

In the Fahli genotype EC 318954 the Na^+: K^+ ratio in the roots under salinity treatments declined as compared to control. Thus, the high uptake of K^+ ions in the roots and low

Na^+: K^+ ratio is the mechanism similar to certain halophytes to cope with increased salinity conditions. Thus, EC 329299 and EC 318954 had similar mechanisms developed for the translocation of excess salts from the shoot region to the roots.

The genotypes T 5-90I-1, EC 4017103 and ISH 8020B had high K^+ content in the shoot across salinity treatments as compared to control, indicating their sensitivity and low tolerance level to salinity conditions due to their failure to exclude Na^+ from the actively growing tissue. These genotypes had high Na^+: K^+ ratio in the shoot indicating their low levels of tolerance to saline conditions and their inability to regulate and control ion transport.

Individual plants differed in their capacity to regulate and control ion transport and accumulation. Thus to improve salt tolerance in *T. alexandrinum* efforts can be made for selecting plants with low Na^+ in the shoot and high K^+ in the roots. Winter and Lauchli (1982) reported that in *T. alexandrinum* leaf production is less affected by moderate salinity than stem production due to translocation of Na^+ from the leaves to other plant parts for exclusion/accumulation. Winter and Lauchli (1982) found that *T. pratense* translocates Na+ and Cl- linearly into stems and leaves, whereas low foliar Na+ and Cl- contents in *T. alexandrinum* suggest some mechanisms that control the ion distribution in the different plant parts. The most striking difference in ion distribution between the two species is in the leaves, where K+ content at all salt regimes was much higher in *T. pratense* than in *T. alexandrinum.* However, in present experiment the whole shoot portion has been taken for ion estimation, hence, the values for Na^+ and K^+ might have been the average of the total Na^+ and K^+ content in the leaves, stem and internodes parts of the plants.

Ashraf (1989) has reported decreased dry weight of Berseem with increasing salinity except in tolerant cultivars like 'Faisalabad late' which had higher shoot and root Na^+ and Cl^- contents than the other cultivars at all salinity levels. However, he did not find consistent correlation between biomass and ion content.

Noble and Shannon (1990) reported that control of Cl^- uptake in Lucerne (*Medicago sativa*) and white clover (*Trifolium repens*) was found to be effective criterion for its *in vitro* selection for salt tolerance. Rogers et al., (1997) proposed further that it is possible to increase levels of salt tolerance in white clover by selecting for low shoot Cl^- concentrations under early stages of exposure to NaCl (i.e. day 4 or 5).

Selection of plant types from the target environment for tolerance to abiotic stress is important in development of salt tolerant lines of any crop. Shakur et al. (1986) used natural populations of *T. repens* growing on saline and non-saline sites for the development of salt tolerance in cultivars, and found that plants from salt marsh sites showed high or very high salt tolerance with relatively vigorous root growth in 150-200 mM NaCl. Hence, in this context selection of plant growing in vitro under saline condition appears quite helpful and has more precision in development of salt tolerant lines.

C. Effect of secondary salinization

C. 1 Plant Growth

Five genotypes were evaluated in sand culture conditions up to 60 days of growth under secondary salinization. Genotype ISH 8020B attained maximum height at 0.50% salinity whereas EC 407709 attained maximum height at 0.75% and 1.0% salinity. The differences for height of the plants across the genotypes under three salinity treatments were highly significant.

In majority cases the number of leaves was maximum under control condition which gradually decreased with increasing salinity in all the genotypes. Maximum leaves were observed in the tetraploid genotype T 45-1, which is a tetraploid genotype. Leaf length was also maximum in control condition and was least affected in EC 407709. Under stressed condition at 0.50% and 0.75% salinity ISB 8020 B and EC 407709 showed large leaves. Amongst crop plants Berseem is considered moderately salt tolerant and leaf production in the crop under stressed condition is less affected by moderate salinity than stem production because of low foliar salt levels under moderate salinity (Lessani and Marschner, 1978; Greenway and Munns, 1980). Leaf width was not much affected under saline condition. The differences in number of leaves in the plants growing at different salinity levels were found to be highly significant. Differences for leaf length in the genotypes under different salinity treatments found to be highly significant. Biomass of the plants gradually decreased with increasing salinity. ISH 8020B yielded maximum at 0.50% salinity. However, at 0.75% salinity ISH 8020B and EC 407709 yielded more than the other genotypes. At 1.0% salinity also EC 407709 yielded maximum. Trend for shoot and root biomass when considered separately also showed the same trend. Analysis of variance revealed that the difference for root and shoot biomass under different salinity levels was highly significant.

C. 2. Isozyme analysis

Enzyme activity was studied in 60 day old plants of the five genotypes growing in sand culture.

The SOD banding pattern did not show any specific band appearing or disappearing in saline condition in genotypes EC 329299 and EC 318954. However, SOD band 2 was observed only in stressed plants of the other three genotypes i.e. T 45-1, EC 407709 and ISH 8020B. The band 1 in the genotypes EC 329299 and EC 407709 had greater intensity in all the three salinity treatments as compared to control. Band 3 and 4 had greater intensity at higher salinity in the genotype T 45-1 and ISH 8020B. Thus, the bands 1, 2 and 3 had significance for salt tolerance against secondary salinization.

Esterase band 10 in EC 329299, band 6 and 10 in ISH 8020B and band 4 in EC 318954 and T 45-1 were specific to high salinity conditions only. Intensity of band 8 increased under saline conditions as compared to control similarly band 9 in the genotypes EC 329299, EC 318954 and EC 407709 had increased intensity under salt stress. Further, all the three genotypes were found to possess tolerance to salinity. Hence, increased activity of this band has greater significance related to salinity tolerance.

C. 3. Native protein

Band 9 and 11 in genotype EC 329299 and band 4 and 16 in EC 318954 were salinity specific. Band 5 and 8 were present in stressed plants only in genotype EC 407709. Similarly band 1, 4 and 14 were present only in stressed plants in the genotype ISH 8020B and T 45-1 in addition to band 4 in ISH 8020B and band 5 in T 45-1 which were present only in stress condition. These results clearly indicated that certain bands appeared under stress conditions which might have rendered tolerance to the plants to cope with stress. But these bands differed in molecular weight. High molecular weight bands 1, 4 and 5 appeared in some genotypes whereas in others low molecular weight bands 9, 11, 14 and 16 appeared under stress. The stress proteins are reported to synthesize *de nova* in response to salt stress or may be present constitutively at low concentrations and increase when plants are exposed to salt stress (Pareek et al., 1997). The genotypes under study represented a diverse group and it is quite likely that different genotypes have different proteins synthesized as a defense mechanism. These genotypes have shown some degree of tolerance at different stages of growth, hence, these bands are important for further molecular studies.

Intensity of band 10 in the genotype EC 329299, EC 318954 and EC 407709 increased under saline conditions whereas in the genotype T 45-1 it remained more or less same. Band No. 12 in the genotypes EC 329299, EC 407709 and ISH 8020B had greater intensity in the salinity treatments. Band 13 in the genotypes EC 329299, T 45-1, EC 407709 and ISH 8020B had increased intensity in the salinity treatments as compared to control. Band 14 also had higher intensity in salinity conditions in the genotypes ISH 8020B, EC 407709, T 45-1.

Higher contents of soluble proteins have been found in salt tolerant than in salt sensitive Sunflower (Ashraf et al., 1995), Finger millet (Uma et al., 1995) and rice (Pareek et al. 1997). Intensity of protein related to stress is reported to increase when plants are exposed to salt stress (Pareek et al., 1997). These proteins sometimes play a role in osmotic adjustment (Singh, 1987). For example a 26Kda protein 'osmotin' was characterized to be salt induced protein in tobacco (Singh et al., 1987). Two 26Kda polypeptides, not immunologically related to osmotin, identified as germin, were found to increase in response to slat stress in barley (Hurkman et al., 1991). Uma et al. (1995) found 54Kda and 23-24Kda proteins responsible for salt or drought tolerance in finger millet (*Eleusine coracana*). Yamada et al. (2002) found a specific protein allene oxide cyclase (AOC named as mangrin) responsible for enhanced salt tolerance in mangrove, *Bruguiera sexangula*.

In the present investigation some proteins showed higher concentrations in stress condition whereas some disappeared. Genotypic response was also different. Differential response of genotypes to increased salinity owing to different mechanism of salt tolerance is also reported in lentil (Ashraf and Waheed, 1993) wherein leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance. Similarly, salt tolerant and salt sensitive accessions of Safflower did not differ significantly in leaf soluble proteins (Ashraf and Fatima, 1995). Pareek et al. (1997) suggested that stress proteins could be used as important molecular markers for improvement of salt tolerance using genetic engineering techniques.

C. 4. SDS PAGE Protein

Sodium dodecylsulphate polyacrylamide (SDS) gel electrophoresis analysis on 60 days old plants of five genotypes growing in sand culture revealed 23 protein bands ranging between 205 Kd to 20 Kd of which 11 bands were monomorphic. Polymorphism was

observed for 12 bands (i.e. band no. 1, 3, 4, 5, 8, 9, 10, 16, 17, 19, 20, 21) which accounted for genotypic variation as well as variation between stressed and non-stressed plants.

Most of the bands were represented commonly in stressed and non-stressed plants of the same genotype; however, following few bands appeared in some genotypes under saline condition only:

Band No.16, 19 and 20 in EC 329299, band 9, 16, 17, 20, and 21 in EC 318954, band 8 and 19 in T 45-1 and band 16 in ISH 8020B were salinity specific. Thus, band 16 (37.4 Kd) and 19 (29Kd) appeared to be more salinity specific, of which band 19 was very near to the 26Kd protein 'osmotin' characterized to be salt induced protein in tobacco (Singh et al., 1987)

D. *In vitro* callusing and embryo culture response

In vitro response of various explants to varying levels of salinity (0.25%, 0.50%, and 0.75%) in callus inducing media L_2 were carried out in 3 different genotypes representing three ecotypes of *T. alexandrinum* i.e., Wardan (Mescavi), EC 318954 (Fahli) and EC 329299 (Saidi). Experiments were carried out to study the interaction effect of various factors such as genotype, explants source, media, levels of salinity etc.

D.1. Callusing response

Callus induction from at 0.25% salinity: Hypocotyl explants responded better (80% response) in saline condition as compared to control but genotypic differences were not much marked. The callus was globular, pale green to pale yellow and attained an average size comparable to the callus growing under control condition.

Callus induction at 0.50% salinity: At 0.50% salinity only 10 to 20 % of the petiole and hypocotyls explants developed into globular/compact and pale yellow callus in genotypes EC 318954 and EC 329299. The growth of callus was poor. However, in genotype Wardan at 0.50% salinity, 60% of the petiole explants and 30 % hypocotyl explants developed into globular to fragile and pale yellow to green callus.

Callus induction at 0.75% salinity: At 0.75% salinity only 30%, 60% and 70% petiole explants of the genotypes EC 318954, EC 329299 and Wardan respectively developed into compact callus. The callus was mostly pale yellow to green with poor growth. However, in Wardan, the growth rate of the callus was satisfactory. Response of hypocotyl explants was comparatively poor and 20 to 40% explants responded to

callusing. Thus, hypocotyl explants at low salinity, petiole and hypocotyl explants at moderate salinity and petiole explants at high salinity responded well. The sensitivity of the explants to NaCl has been reported to be in the order radicle>hypocotyls> cotyledon in cotton (Wang et al., 1991).

Response of callus, induced in saline condition, to higher salinity: The petiole and hypocotyl derived callus induced in 0.25% salinity when transferred to callusing medium at 0.50% salinity responded positively and proliferated into globular, compact, pale yellow to green callus in majority of cultures. However, when transferred to shoot inducing LSP3 medium, the response was slow but most of the calli showed proliferation. The petiole-derived calli developed at 0.50% salinity, when transferred to 0.75% salinity also responded positively. Such calli responded well to LSP_3 medium also. The callus was mostly globular and fragile, pale yellow to green and the growth rate of the callus was good. The petiole-derived calli developed at 0.75% salinity when transferred to LSP_3 medium showed comparatively less response and 37.5% of the callus responded positively to regenerating media. Hypocotyl derived calli developed at 0.75% salinity were transferred to LSP_3 and 75.0% of the callus responded positively to regenerating media. Thus, calli developed even up to 0.75% salinity and transferred to 0.75% salinity levels showed callus proliferation ranging from good to medium.

***In vitro* response of callus to further increased salinity:** All the genotypes as well as calli derived from different explants developed at various salinity levels responded positively to increased salinity treatment even after second subculture. Callus proliferation was good in SEIM, LSe and LSP3 media also. Prolonging the selection process *in vitro* in rice has been reported to improve the likelihood of regenerating plants with improved salt tolerance (Winicov 1996). Successive subcultures in Lucerne into media of progressively higher salinity resulted in regenerants, which were more salt tolerant than the original (Chaudhary et al., 1996)

D.2. *In vitro* embryo culture response

Fertilized flowers of the three ecotypes of *Trifolium* i.e., EC 329299 (Saidi), EC 318954 (Fahli) and Wardan (Mescavi) were brought to laboratory. The embryos at cotyledonary stage were excised, surface sterilized under aseptic conditions and inoculated on MS basal media supplemented with 0.3% Kinetin and further supplemented with 0.25%, 0.50% and 0.75% NaCl.

nitial **embryo culture response:** *In vitro* germination of embryo of the three genotypes anged between 69.5 to 85.7% in control whereas its response under 0.25% salinity was naximum in Wardan wherein 83.3% embryos germinated as compared to 58.6% in Fahli and Saidi. At higher salinity also maximum germination was noticed in Wardan i.e. 27.3% at 0.75% as compared to 12% in Fahli and Saidi. .

Mortality under control condition ranged from 13.8 to 18.2% among three genotypes. However, high degree of mortality was observed in Fahli and Saidi genotypes under stressed condition. Mortality in these genotypes was up to 100% at 0.75%. In case of Wardan mortality was quite less as compared to other two genotypes at 0.25%, 0.50% and 0.75% salinity 32, 59.1 and 66.7% of the plants degenerated. These results indicated that tolerance against salt stress in embryo development is much higher in Mescavi ecotype as compared to Fahli and Saidi. However, screening study established better tolerance in Saidi and Fahli. This might be due to the fact that Saidi and Fahli are self compatible genotypes and have less or no intragenotypic variation for salt tolerance whereas Wardan may be a population of many phenotypically similar but genetically different plants which must have given a chance of *in vitro* selection. Thus, embryo culture can be used as rapid *in vitro* screening procedure. Rapid *in vitro* screening of 14 cultivars of *Triticum aestivum* using excised mature embryos for salt tolerance was also reported by Diaz et al. (1995).

The surviving plants were transformed to MS media and RL media supplemented with required amount of NaCl for 0.25%, 0.50% and 0.75% salinity treatments. The plants were transferred to the same level of treatment in which they were growing. The sub cultured seedlings of EC 318954 survived at 0.25% salinity and 5 to 6 leaves emerged whereas at 0.50% salinity none of the plants survived. The most of sub cultured seedlings of EC 329299 degenerated by 20^{th} day both at 0.25% and 0.50% salinity whereas most of the subcultured seedlings of Wardan survived at 0.25% salinity and its growth was also good. At 0.50% salinity one plant could survive whereas all the plants died at 0.75% salinity.

E. Molecular characterization of selected genotypes

RAPD study was carried out using 30 decamer Random primers (Operon Technologies, Inc.) Out of 30 decamer oligonucleotide primers used for amplification of Genomic DNA of 8 different genotypes of Berseem, 7 did not react. The rest 23 primers generated one or more unambiguously scorable bands. Number of amplification products varied with primer N-20 producing maximum 14 bands whereas primer OPR-06 and OPF-6 produced

minimum 6 distinct bands. The 23 primers in total yielded 216 bands of which 71 bands were polymorphic (32.87%) and 145 bands (67.12%) were monomorphic. The primer OPR-06 and AK-14 produced 6 and 9 distinct bands respectively all of which were monomorphic whereas the primer AB-10 produced 8 bands and all were polymorphic.

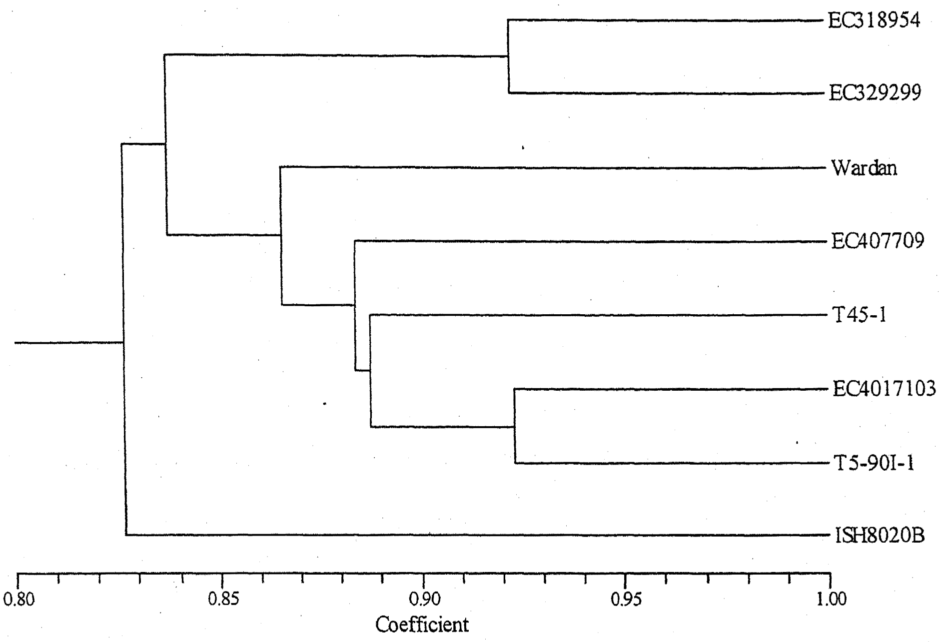
Cluster analysis based on 216 bands revealed 82 to 92% similarity among the eight genotypes (Fig 11). Fahli genotype EC 318954 showed 92.27% similarity with Saidi genotype EC 329299. Mescavi genotypes along with exotic, indigenous and tetraploid showed 83.74 similarities among the genotypes like Wardan, EC 407709, T 45-1, EC-4017103 and T 5-90I-1. EC 407709, T 45-1, EC 4017103 and T 5-90I-1 exhibited 86.55% within group similarity. Group of three genotypes T 45-1, EC 4017103 and T 5-90I-1 showed 88.42% similarity. Genotype ISH 8020B which is a cross between *T. alexandrinum* with *T. apertum* showed least similarity (82.69%) with other genotypes thus, separating out it from the other genotypes. RAPD bands no. 3, 4, 5, 6 and 8 (AB-10), 4 (V-02) and 1 (AB-5 and B-14) were specific to genotypes EC 318954 and EC 329299. These genotypes have also been found to possess high degree of salinity tolerance. As these are different ecotypes also, they differ with other genotypes for many traits such as regeneration potential and flowering time. Hence, this can be further investigated whether these bands are linked to salinity or not.

F. Identification of Genotypes

Identification of genotypes for salt tolerance is one of the most important outcome of the study. As the results have clearly established the genotypic differences for response to salinity at different levels, the identification was done on two criteria i.e. i) the best performing genotypes based on absolute values of traits under observation ii) genotypes showing least reduction under saline condition with respect to control as these genotypes can be good source of resistance genes for different traits.

On the basis of the percent reduction in germination over control genotype such as ISH 34/11, EC 407709 and Penta 99-1 were tolerant for germination at different salinity levels. The genotypes Penta 99, EC 329299 and EC 318954 were found to be tolerant upto 0.50% salinity level but at higher salinity level i.e. 0.75% these recorded more than 50% reduction in germination. For shoot growth among 20 days old seedling, genotypes Penta 99, ISH 34/49 and EC 407709 were most tolerant to varying levels of salinity. For root growth genotypes EC 407709, ISH 34/49, Penta 99 and EC 400977 were comparatively tolerant to other genotypes at varying salinity levels. EC 407709, EC

Fig 11. Dendrogram based on RAPD banding pattern showing similarity among genotypes of Egyptian clover.



Similarity matrix of Berseem genotypes based on RAPD banding pattern

	EC 318954	EC 329299	Wardan	EC 407709	T 45-1	EC 4017103	T 5-90I-1	ISH 8020B
EC 318954	1.000							
EC 329299	0.923	1.000						
Wardan	0.827	0.845	1.000					
EC 407709	0.798	0.817	0.864	1.000				
T 45-1	0.836	0.854	0.863	0.885	1.000			
EC 4017103	0.858	0.829	0.866	0.878	0.897	1.000		
T 5-90I-1	0.860	0.850	0.868	0.890	0.878	0.923	1.000	
ISH 8020B	0.808	0.800	0.806	0.834	0.834	0.856	0.849	1.000

0977, EC 401711 and ISH 32/8/1 had minimum reduction in number of leaves at all inity levels indicating their relative tolerance compared to the other genotypes. enotypes ISH 34/49, Penta 99-1, Penta 99 and T 9-90FM were least affected due to rying levels of salinity for biomass (Table 36).

n the basis of percent reduction over control in 45 day old plants also genotypes were entified. For shoot length, at 0.25% salinity ISH 5050Y had the least reduction in shoot ngth (2.5%) over control whereas ISH 32/34/1, T 5-90I-1, ISH 34/49 and T 5-90-I corded around 25% reduction. At 0.50% salinity T 5-90-I, ISH 34/49, T 5-90I-1 and T -90FM recorded minimum (50%) reduction in shoot length over control whereas the ther genotypes recorded 70 to 90% reduction in shoot length. At 0.75% salinity ISH 050B had the least (60%) reduction, EC 407709 and ISH 32/34/1 had 65% reduction whereas the other genotypes recorded 75 to 90% reduction in shoot length. EC 329299 and EC 318954 had low reduction in root length upto 0.50% salinity whereas EC 407709 had the least reduction at higher salinity as compared to other genotypes. Genotypes EC 329299, EC 318954, Raj Bundi and ISH 32/34/1 recorded less reduction in number of leaves upto 0.50% salinity whereas EC 407709 recorded least reduction at higher level of salinity. For biomass genotypes EC 407709, ISH 34/41 and ISH 32/34/1 had almost minimum reduction in biomass yield at all salinity levels (around 50%) indicating less sensitivity to increasing salinity. Genotype ISH 34/49, Raj Bundi and T 9-90FM had low reduction upto 0.50% salinity. Thus these genotypes can be source of genes of resistance for different traits (Table 37).

On the basis of performance the genotypes were ranked. The top five best performers were given score of 5 to 1 in order of their ranking i.e. top ranker given score 5 and fifth from top given score of 1. Thus, hereunder is presented the best performing genotypes found common for 20 and 45 days of exposure to salinity different traits at different salinity levels in order of their ranking (Table 38-39).

Germination: ISH 34/11, EC 407709, EC 318954, EC 329299, ISH 34/49.

Shoot length: EC 407709, EC 329299, EC 401711.

Root growth: EC 407709, EC 318954, EC 329299, ISH 8020B.

No. of leaves: EC 407709, EC 329299, ISH 34/8B.

Weight of plants: T 9-90FM, EC 407709, EC 329299, EC 318954, T 5-90-I, ISH 34/8B

On the basis of total score of various traits at different salinity levels EC 407709, EC 318954 and EC 329299 were found top ranking.

Table 36. Percent reduction over control in 20 day old seedlings

Genotype	% germination			Shoot length			Root length			No of leaves			Weight of plant		
	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%
EC 329299	4.59	27.56	71.73	15.29	53.62	80.84	10.16	61.78	84.67	9.48	37.07	66.38	-2.08	39.58	72.92
EC 318954	6.25	9.72	60.07	20.68	59.50	77.92	18.04	60.20	88.82	22.41	55.17	66.38	45.71	68.57	14.29
Wardan	11.11	51.11	75.56	32.14	61.43	76.00	25.67	75.87	90.95	27.27	46.97	54.55	9.09	45.45	63.64
EC 407709	12.28	14.04	21.05	29.26	9.09	14.20	25.43	14.29	27.71	9.78	17.39	3.26	33.33	0.00	12.12
EC400976	14.81	44.44	85.19	23.88	69.46	81.66	37.36	61.10	78.02	4.65	30.23	65.12	8.82	32.35	70.59
EC 508311	76.19	83.33	100.00	57.65	63.11	100.00	51.33	29.26	100.00	39.52	39.52	100.00	46.67	46.67	100.00
EC4017103	50.58	76.74	85.47	52.78	76.33	73.96	50.88	78.39	76.42	38.14	38.14	38.14	50.00	56.25	75.00
EC400977	7.19	85.03	82.04	59.80	78.75	65.56	49.28	73.92	62.41	23.08	23.08	23.08	44.44	44.44	55.56
EC401711	34.91	70.41	88.17	33.36	37.64	87.27	42.39	48.52	83.66	-10.41	23.86	23.86	0.00	11.76	76.47
ISH 34/49	5.26	26.32	64.91	1.77	33.63	25.66	20.19	30.94	54.72	23.61	26.39	5.56	-7.14	-7.14	7.14
ISH 34/41	37.04	48.15	51.85	32.80	62.40	77.68	74.49	83.67	81.63	23.86	52.27	62.50	48.15	37.04	55.56
ISH 34/11	-1.79	19.64	23.21	22.11	31.58	55.79	45.16	47.58	75.81	54.43	58.23	58.23	9.09	9.09	18.18
Penta 99	9.52	7.14	54.76	-27.59	25.29	59.77	24.55	44.09	67.95	21.21	54.55	37.88	10.53	5.26	10.53
Raj Bundi	18.60	37.21	51.16	15.04	57.52	58.41	13.65	31.97	68.81	13.04	56.52	44.93	15.00	15.00	5.00
Penta 99-1	14.35	35.19	37.50	16.48	48.52	50.00	23.12	5.59	73.80	18.28	54.98	38.61	0.00	19.05	-600.00
ES 99	20.00	48.00	74.00	26.92	44.02	67.78	10.37	29.17	80.67	33.50	50.00	50.00	36.36	40.91	59.09
ISH 32/8/1	43.14	62.75	76.47	24.93	40.42	55.49	59.74	66.23	84.85	0.90	9.91	9.91	6.67	26.67	53.33
Wardan S2	28.85	55.77	67.31	57.58	49.16	83.63	74.80	41.36	84.98	48.88	33.77	63.33	37.04	11.11	51.85
ISH 26/50/7	54.55	78.18	85.45	26.82	73.58	89.39	49.42	84.81	90.65	36.64	66.97	69.97	39.29	39.29	75.00
ISH 32/34/1	17.39	76.09	91.30	17.92	24.64	89.68	39.40	16.53	86.64	33.42	17.43	62.45	4.00	-20.00	72.00
Multi-98-45	39.58	79.17	85.42	56.93	81.33	86.00	51.68	80.97	91.22	63.94	63.94	63.94	30.77	73.08	80.77
ISH 34/5/1	15.22	56.52	84.78	40.32	62.40	87.60	37.66	40.63	87.01	48.14	60.02	63.99	20.00	15.00	65.00
Raj 49/50	81.48	100.00	100.00	77.07	100.00	100.00	74.81	100.00	100.00	49.92	100.00	100.00	52.94	100.00	100.00
T 5-90I-1	47.17	88.68	88.68	57.44	91.62	61.21	26.59	95.92	91.60	55.31	63.37	63.37	-31.82	81.82	68.18
T 45-1	46.67	83.33	85.00	49.52	54.65	80.91	49.18	79.08	86.66	35.84	42.81	67.81	20.00	32.00	60.00
T 44-4	39.22	80.39	80.39	42.67	82.73	79.53	79.05	95.14	93.32	37.93	68.91	65.49	42.86	75.51	81.63
ISH 8020B	30.51	52.54	74.58	38.62	61.29	71.39	20.13	69.68	89.94	32.51	44.97	51.20	40.00	58.18	70.91
ISH 8020Y	38.18	67.27	80.00	55.28	68.26	86.74	59.97	82.47	94.67	16.72	46.65	69.97	42.11	57.89	68.42
ISH 5050B	14.29	64.29	58.93	32.11	59.44	80.94	57.93	79.36	94.93	15.73	60.47	76.28	12.50	37.50	56.25
ISH 5050Y	16.67	57.41	90.74	31.63	53.94	80.64	23.84	57.34	91.89	16.63	48.30	69.94	24.00	28.00	64.00
ISH 34/8B	37.78	53.33	68.89	39.69	62.12	88.67	48.18	53.87	91.61	34.31	23.16	74.27	41.86	27.91	83.72
ISH 34/8Y	35.29	66.67	66.67	2.26	44.64	70.34	22.60	60.21	76.11	8.83	41.17	70.58	-12.12	36.36	51.52
T 5-90-I	30.51	62.71	79.66	32.06	62.52	84.42	17.99	64.31	87.62	15.10	57.60	66.70	-4.76	23.81	71.43
T-9-90FM	10.53	35.09	82.46	21.65	73.23	87.45	9.15	50.47	91.45	-0.09	70.58	73.50	2.17	43.48	-139.13

Table 37. Percent reduction over control in 45 day old plants.

Genotype	Shoot Length			Root Length			No. of leaves			Weight of plant		
	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%
EC 329299	42.9	72.4	89.0	-5.6	72.8	89.4	23.8	39.3	85.7	54.2	71.1	88.2
EC 318954	31.9	64.8	81.4	41.8	40.3	90.0	27.3	31.8	72.7	58.2	63.3	81.1
Wardan	61.6	63.5	82.7	68.7	87.5	96.2	30.8	23.1	65.4	45.6	56.0	58.7
EC 407709	62.0	71.1	65.9	74.2	89.9	68.2	55.6	48.1	33.3	56.3	59.4	51.4
EC 400976	62.3	78.2	100.0	77.9	85.0	100.0	43.3	60.0	100.0	48.4	71.5	100.0
EC 508311	65.8	100.0	100.0	86.2	100.0	100.0	60.0	100.0	100.0	67.1	100.0	100.0
EC 4017103	79.6	82.6	100.0	94.4	97.6	100.0	73.3	80.0	100.0	86.6	83.4	100.0
EC 400977	91.1	94.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	44.3	89.2	100.0
EC 401711	74.1	100.0	100.0	57.6	100.0	100.0	33.3	100.0	100.0	71.5	100.0	100.0
ISH 34/49	20.2	50.5	100.0	71.2	82.3	100.0	18.8	65.6	100.0	11.6	30.0	100.0
ISH 34/41	44.5	66.8	76.1	79.2	94.4	100.0	28.8	56.3	62.5	28.6	35.2	41.3
ISH 34/11	39.9	65.9	81.0	76.4	91.2	100.0	36.4	56.4	72.7	55.6	63.8	67.0
Penta 99	27.1	58.3	100.0	54.8	79.3	100.0	25.0	60.0	100.0	24.0	47.5	100.0
Raj Bundi	30.5	61.2	100.0	72.1	79.7	100.0	20.0	35.0	100.0	27.4	36.5	100.0
Penta 99-1	41.1	100.0	100.0	38.5	100.0	100.0	44.4	100.0	100.0	0.3	100.0	100.0
ES 99	47.4	100.0	100.0	75.4	100.0	100.0	55.0	100.0	100.0	49.5	100.0	100.0
ISH 32/8/1	35.1	57.9	100.0	79.7	82.4	100.0	66.7	66.7	100.0	42.2	47.4	100.0
Wardan S2	39.4	87.1	100.0	54.8	78.5	100.0	33.3	80.0	100.0	12.7	84.3	100.0
ISH 26/50/7	62.8	73.5	87.8	71.8	100.0	100.0	55.9	76.5	100.0	33.4	54.3	87.9
ISH 32/34/1	15.9	59.0	68.3	23.3	75.6	92.2	4.5	56.4	100.0	-7.7	32.7	49.4
Multi-98-45	57.3	75.5	100.0	57.1	75.6	100.0	35.0	75.0	100.0	34.8	46.7	100.0
ISH 34/5/1	52.5	75.4	100.0	70.9	73.2	100.0	30.0	50.0	100.0	2.4	22.2	100.0
Raj 49/50	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0
T 44-4	62.3	94.3	93.2	89.1	100.0	100.0	50.0	100.0	100.0	67.3	92.9	92.2
T 45-1	76.4	75.9	79.6	89.7	87.4	94.6	62.5	43.8	43.8	64.2	44.2	48.9
T 5-90I-1	17.7	56.5	72.2	52.9	89.3	85.3	18.2	45.5	72.7	-57.5	33.8	54.1
ISH 8020B	56.3	71.1	78.4	50.7	79.6	92.3	40.0	58.8	62.5	54.6	70.9	76.4
ISH 5050B	34.9	71.4	59.4	73.6	89.1	86.5	26.9	53.8	65.4	15.2	48.2	25.7
ISH 5050Y	2.5	100.0	100.0	55.1	100.0	100.0	20.0	100.0	100.0	22.1	100.0	100.0
ISH 34/8B	49.1	74.6	84.7	66.2	85.8	95.3	26.5	61.2	82.4	-2.7	56.3	70.1
ISH 34/8Y	46.3	77.9	78.2	47.7	87.9	91.9	28.9	76.3	68.4	26.7	63.7	75.0
T 5-90-I	25.8	45.8	76.5	58.9	81.8	89.6	35.3	25.9	73.5	-5.4	-58.7	64.5
T-9-90FM	48.3	56.7	81.3	46.5	76.8	100.0	36.5	61.2	82.4	14.4	20.2	81.3

Table 38. Score card of genotypes for early seedling vigour.

Genotypes	Germination			Shoot Length			Root Length			No. of Leaves			Weight of Plant			Total
	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	
EC 329299	4	1		2	3		4		1	2	2		5			24
EC 318954	3	5	1	3	1		5	3							3	24
Wardan																
EC 407709		4	5	5	5	5	1	5	5	5	5	5		5	2	57
EC400976				4			3	2	4	4	4	3	3	3		30
EC 508311																
EC4017103																
EC400977																
EC401711					4								1	4		9
ISH 34/49	2	2				4			3			4				15
ISH 34/41			2													2
ISH 34/11	5	3	4													12
Penta 99															4	4
Raj Bundi						2									1	3
Penta 99-1			3					1								4
ES 99					2	3		4				2				11
ISH 32/8/1												1				1
Wardan S2																
ISH 26/50/7																
ISH 32/34/1																
Multi-98-45																
ISH 34/5/1																
Raj 49/50																
T 5-90I-1																
T 45-1																
T 44-4																
ISH 8020B						1	2									3
ISH 8020Y																
ISH 5050B																
ISH 5050Y																
ISH 34/8B											3			1		4
ISH 34/8Y									2	1	1					4
T 5-90-I													2	2		4
T-9-90FM	1			1						3			4		5	14

Table 39. Score card of genotypes in 45 days of plant growth													
Genotypes	Shoot Length			Root Length			No. of Leaves			Weight of Plant			Total
	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	0.25%	0.50%	0.75%	
EC 329299	4	4		5	5	3	5	4			2		32
EC 318954	5	5	5	4	4	4	4	5	2	1	3	2	44
Wardan								2					2
EC 407709			4		1	5			5			5	20
EC 400976													3
EC 508311													
EC 4017103													
EC 400977										4			4
EC 401711				2									2
ISH 34/49	1	2					2						5
ISH 34/41													
ISH 34/11													
Penta 99													
Raj Bundi													
Penta 99-1													
ES 99													
ISH 32/8/1													
Wardan S2													
ISH 26/50/7													
ISH 32/34/1													
Multi-98-45													
ISH 34/5/1													
Raj 49/50													
T 44-4													
T 45-1		1	1					1	4			4	11
T 5-90I-1													
ISH 8020B			2	3	3	2			3				13
ISH 8020Y													
ISH 5050B			3									3	6
ISH 5050Y													
ISH 34/8B	2						1			2			5
ISH 34/8Y							3		1		1		5
T 5-90-I	3	3				1		3		3	5	1	19
T-9-90FM				1	2					5	4		12

SUMMARY

SUMMARY

Soil salinity is one of the major abiotic stresses which significantly affect crop productivity throughout the world. An FAO study in 1989 estimated that up to 7% of the world's land area is salt affected. The rate at which the salinity problem is increasing, it is important to identify salt resistant varieties/cultivars of economically important crops. Promising approaches would be either to use variation existing in wild relatives or to use tissue culture techniques for selection of mutation for salt resistance. Further, most of the earlier studies on salt tolerance have been restricted to evaluation of a few genotypes under saline condition, whereas it is being realized that variation existing at genotypic level as well as the variation at intra-genotypic level in the crops having heterozygous background should also be exploited.

Looking into the increasing demands for fodder and shrinking area under forage cultivation the problem soils are the only area where from forage production can be given a boost. Among the various forages Berseem is cultivated in 2 m ha area. Considering its high production potential and wide adaptability to diverse growing conditions, the crops adaptations to saline/sodic conditions needs to be evaluated. Thus, the study was planned with following objectives:

- *In vitro* screening of *Trifolium alexandrinum* lines for varying levels of salt concentration.
- Studies on biochemical attributes related to differential response under control and stress conditions.
- Studies on salinity tolerance under *in vitro* culture conditions at different salt concentrations.

The study involved 34 genotypes of *T. alexandrinum* representing three ecotypes i.e. Mescavi, Saidi and Fahli. The accessions also represented tetraploids and interspecific hybrid progenies. The genotypes were screened *in vitro* for germination, survival, seedling vigour and growth under three treatments i.e. 0.25%(EC 4.8dSm-1), 0.50%(EC 8.1dSm-1), 0.75% (EC 11.3dSm-1) in MS media and observations recorded on on 20th as well as 45th day of growth for germination, root/shoot length, number of leaves, plant weight. Isozyme studies in seedlings growing *in vitro* was also conducted on 20 days old seedlings for PRX, Est and SOD. Among selected genotypes protein banding pattern (native/SDS), Na, K ion estimation was also observed. Effect of secondary salinization in sand culture on 5 genotypes at 0.50 %(EC 8.1dSm-1), 0.75% (EC 11.3dSm-1) 1.0 %(EC

14.6dSm-1) salinity was conducted and data recorded on 60 day old plants. *In vitro* callusing response of 3 genotypes, 2 explants (hypocotyls and petiole) at 0.25%(EC 4.8), 0.50%(EC 8.1), 0.75% (EC 11.3) in L2 media was observed along with their sub culturing response in higher salinity. *In vitro* embryo culture response of 3 genotypes at cotyledonary stage at 0.25 %(EC 4.8), 0.50 %(EC 8.1), 0.75% (EC 11.3) salinity in embryo culture media was also observed.

***In vitro* germination, survival, seedling vigour and growth.**

The effect of saline environment was manifested in reduced and delay in germination. However, the degree of adverse effect varied with genotypes and salinity levels. Genotypes like EC 318954 showed at par germination to the control conditions at 0.25% and 0.50% salinity. Genotypes like EC 407709, ISH 34/11 showed up to 75% germination at 0.75% salinity. EC 508311 was among highly susceptible genotypes, which showed no germination at 0.75% and only 15% germination at 0.25% salinity. EC 4017103, EC 401711, ISH 26/50/7, Multi-98-45, Raj 49/50 also belonged to susceptible group showing drastic reduction in germination even at low salinity stress. Genotypes like EC 400977, ISH 34/49, ISH 34/41, Penta 99-1, Raj Bundi, ES 99, ISH 32/8/1, Wardan S2, T 44- 4, T 45-1, ISH 8020B, ISH 34/8B, ISH 34/8Y, T 5-90-I, ISH 8020Y, ISH 5050 B, T 9-90-FM, showed moderate tolerance and gradual reduction in germination was observed with increasing salinity. Genotypes like ISH 32/34/1, ISH 34/5/1, T 5-90I-1 and ISH 5050Y showed better tolerance (little reduction in germination) at low salinity levels but drastic reduction at high salinity was observed. The sensitivity index for germination of seeds was highest for the genotypes EC 508311 (1.39), Raj 49/50 (1.39), ISH 32/34/1 (1.27), T 5-90I-1 (1.23), and E C 401711 (1.23), indicating their susceptibility to high levels of salinity whereas the genotypes EC 407709 (0.29), ISH 34/11 (0.32), Penta 99 (0.52), Raj Bundi (0.71) and ISH 34/41 (0.72) recorded low sensitivity indices and thus tolerant to saline conditions.

Almost all the plants survived up to 45 days at 0.25% salinity; however, prolonged exposure to saline conditions had deleterious effect at higher salinity levels on the plants. In genotypes EC 329299, EC 400977, EC 400976, ISH 34/49, ISH 26/50/7, Multi-98-45, ISH 34/5/1, ISH 8020Y, 10 to 15% mortality was observed at 0.50% salinity whereas 50 to 100% of the seedlings perished at 0.75%. Genotype EC 318954 showed no mortality at 0.25% and low mortality (10 and 20%) at 0.50% and 0.75% salinity respectively. Genotypes Wardan, ISH 32/8/1, Wardan S2, ISH 32/34/1, T 44-4, T 5-90I-1, ISH 5050B,

ISH 34/8B had moderate to high increase in mortality from 0.25 to 0.75% salinity. Genotypes EC 407709, ISH 34/41, ISH 34/11, T 45-1, ISH 8020B, ISH 34/8Y, T 5-90-I, T 9-90-FM were tolerant to prolonged salinity and less than 30% mortality at 0.75% salinity was observed.

Seedling vigour and growth

Tolerance observed at germination, early seedling and the vegetative growth stage is of great importance. Growth of plants on 20th day in saline conditions was found to be highly variable in genotypes showing their heterogeneous nature. The seedlings with reduced or no root growth were considered to be susceptible whereas the seedlings with normal root growth were considered to be tolerant as the development of roots is of primary importance in the stand establishment of any crop/plant.

Sensitivity index for shoot length was highest among genotypes Raj 49/50, EC 508311, ISH 32/34/1 and ISH 26/50/7, thus showing susceptible nature. Genotypes EC 407709 (0.19), ISH 34/49 (0.35), Raj Bundi (0.80), ISH 34/11 (0.77) and Penta 99 (0.82) showed tolerance towards salinity as indicated by low SSI values. It appeared genotypes EC 407709, ISH 34/11 and Penta 99 possessed some mechanism for salt tolerance as far as shoot growth was concerned. Genotype EC 407709 had the maximum growth in both susceptible (2.58 cm) and resistant category of plants (10.72 cm). Selection between and within cultivars is likely to lead increased salt tolerance in this genotype. In the present study also shoot length was reduced and differential response of seedlings in same genotypes was observed indicating chances for intragenotypic selection.

Sensitivity index for root growth was highest in the genotypes EC 508311 (1.20), Raj 49/50 (1.20), ISH 5050B (1.14), ISH 8020Y (1.14) and T 44-4 (1.12) whereas the genotypes EC 407709 (0.33), ISH 34/49 (0.66), EC 400977 (0.75), Penta 99 (0.82) and Raj Bundi (0.83) had minimum reduction in the root length as indicated by their low SSI values. Thus, based on growth and development of roots genotypes EC 508311 and Raj 49/50 were highly sensitive whereas genotypes EC 407709, EC 400977, ISH 34/49, Penta 99 and Raj Bundi were tolerant. Genotype EC 407709 had maximum root growth in both the susceptible (2.81 cm) and resistant category of plants (3.95 cm).

The sensitivity index for number of leaves in the genotypes EC 508311 (1.77), Raj 49/50 (1.77), ISH 5050Y (1.35), ISH 34/8B (1.32) and T 9-90-FM (1.30) were found to be high. The genotypes EC 407709 (0.06), ISH 34/49 (0.10), ISH 32/8/1 (0.18), EC 400977 (0.41) and EC 401711 (0.42) were least sensitive for leaf growth as indicated by their low SSI values. Of these genotypes EC 400977, EC 401711 and ISH 32/8/1 showed low SSI

values in susceptible group of plants also indicating that these genotypes possess lesser intra-genotypic variation. Genotype EC 407709 was least sensitive based on data recorded on resistant category of plants while it was susceptible based on data of susceptible group of plants indicating higher intragenotypic variation for salinity tolerance. This indicates that intragenotypic variation exists in *T. alexandrinum* which can be exploited in selection for salinity tolerant plants.

The sensitivity index was highest in the genotypes EC 508311 (2.68), Raj 49/50 (2.68), ISH 34/8B (2.25), T 44-4 (2.19) and Multi-98-45 (2.17), thus indicating their susceptibility to high levels of salinity, whereas the genotypes Penta 99-1 (–16.09), T 9-90-FM (–3.72), Raj Bundi (0.13), ISH 34/49 (0.19) and Penta 99 (0.28) were found to be least sensitive. Genotypes Penta 99-1 isolation and T 9-90-FM even showed higher biomass at 0.75% salinity than control condition. Thus, the genotypes EC 508311, Raj 49/50, and T 44-4 were sensitive to salinity whereas Penta 99-1, Penta 99, Raj Bundi and ISH 34/49 were least sensitive to high salinity levels for biomass production.

Thus, the present study resulted in identifying different genotypes which showed susceptible, moderately tolerant and highly tolerant nature of the genotypes as indicated by germination, shoot/root growth and total biomass production. For example EC 407709, ISH 34/11, Penta 99, Raj Bundi and ISH 34/41 were tolerant for germination but Penta 99-1, T 9-90FM, Raj Bundi, ISH 34/49 and Penta 99 were tolerant for biomass production in terms of reduction as compared to their respective control. However, based on total biomass production genotypes T 9-90FM, EC 407709, EC 329299, EC 318954 and T 5-90-I emerged most tolerant. Similarly the genotypes EC 407709, EC 318954, EC 329299 and ISH 8020B were found tolerant for growth of roots which can be regarded as the one of the most important criteria in selection for salt tolerance indicating that genes for tolerance at various growth stages are scattered across genotypes and can be used in pyramiding of genes for developing a salt tolerant variety/cultivar. Development of broad based germplasm pools by intercrossing salt tolerant lines of diverse origin followed by mass propagation under highly saline conditions can be a realistic approach.

Single factor analysis of variance for germination, shoot length, root length, number of leaves and plant weight of 20 days old seedling revealed significant differences for all the traits under study under salinity treatments among all the genotypes.

Analysis of variance for germination, shoot length, root length, number of leaves and plant weight revealed significant differences both at genotypic and salinity levels for all

the traits under study. Indicating thereby that both genotypes and different levels of salinity treatments has significant affect on the parameters observed

Biochemical studies

Isozymic banding pattern of SOD (Super Oxide Dismutase), PRX (Peroxidase) and Est. (Esterase) under 3 levels of salinity (0.25%, 0.50% and 0.75%) on 20 day old seedlings showed presence of 4 SOD, 11 PRX and 18 Esterase bands.

SOD banding pattern revealed polymorphism for band no. 4 only which was observed in 0.25% salinity treated plants of EC 4017103, ISH 32/34/1 and 0.50% and 0.75% treated plants in EC 400977 and ISH 34/8B. The frequency of all four bands increased among resistant plants at 0.25% salinity but in the susceptible plants it was almost similar to control. Band 4 in resistant plants growing at 0.75% salinity had 100% band frequency whereas in the susceptible plants the frequency was less compared to control indicating that increased SOD activity provided some tolerance mechanism among resistant plants at low salinity levels.

Peroxidase band 3, 5 and 8 were present only in the susceptible plants under different levels of salt stress among the genotypes EC 4017103, EC 400977, EC 401711, ISH 34/11, ES 99, Wardan S2 and EC 407709 however in EC 329299 and EC 400976 band 3 and 8 were present in both susceptible and resistant plants under salt stress. In the genotypes EC 508311, ISH 32/34/1, Multi 98-45 and ISH 34/5/1 band 8, 10 and 11 were present in stressed plants only. In ISH 5050B band 8 was present only in the resistant plants at 0.25% salinity whereas in ISH 8020Y this band was present in both susceptible and resistant plants as well as under control condition. The frequency of band 2 in the control plants was 32% which reduced in the resistant plants growing at different salinity levels. Contrary to this, band 3 and 8 had substantial increased band frequency in plants growing under stress similarly band 10 and 11 also exhibited increased frequency at 0.50% and 0.75% salinity.

Esterase bands 6, 8 and 9 were observed alone or in combination in the plants growing in saline condition in 11 genotypes. EST band 10, 13, 14 and 16 alone or in combination had increased band intensity in the genotype EC 400976, ISH 34/5/1, Wardan, and EC 329299. Thus there were no specific bands associated with saline conditions across genotypes. However band 6, 8 and 9 might have some significance as these bands appeared only in saline conditions in 11 genotypes.

Biochemical study on eight selected genotypes representing 3 different ecotypes, tetraploid and interspecific hybrids growing in pots was also conducted. The study indicated appearance of native protein bands in response to saline conditions. However, the response was genotype specific with appearance of different mobility bands in different genotypes. The bands appearing only in saline conditions either alone or jointly were band 1, 2 and 3 (Wardan, EC 407709, T 45-1 and ISH 8020B), band 8, 10, 11 and 21 (EC 318954), band 3, 4, 7, 8, 9, 10, 11, 12 and 14 (EC 4017103), in tetraploid genotype T 5-90I-1 band 4, 9, 10, 11 and 15 and band 8 in EC 329299. Certain bands present in control plants were not observed in saline condition e.g. Band 6 and 18 in Wardan, band 11 in ISH 8020B and band 21 in EC 4017103. Certain bands appeared only in low salinity conditions only. In tetraploid genotype T 45-1 band 6 and 13 were present only in the plants growing at 0.25% salinity. Similarly in genotype ISH 8020B band 17 was present only in the plants growing at 0.25% salinity and band 22 were present only at 0.25% and 0.50% salinity level in EC 329299.

As defense mechanism, plants synthesize certain proteins in higher quantity. In the present study, out of total 20 bands observed among different genotypes, band 2 in the genotype EC 329299, band 18 in the genotype EC 318954, band 19 in the genotype T 5-90I-1 and band 5 in the genotype EC 318954, EC 4017103, and T 5-90I-1 had greater intensity at all salinity treatments compared to control indicating their higher concentration in stressed plants.

Some proteins were synthesized in higher quantity only at higher salinity levels as expressed by their darker bands in the protein-banding pattern. For example band 4 in the genotype EC 318954 and T 5-90I-1 had increased intensity at higher salinity whereas in the genotype EC 329299 greater band intensity was observed at 0.25%, 0.50% and control conditions as compared to 0.75% salinity level. Band No.11 had increased band intensity at higher salinity level i.e. 0.75% and 1% whereas at 0.25% 0.50% and control condition it was reduced, in the genotype Wardan.

Contrary to above situation it appeared that synthesis of certain protein was masked in stressed condition that is why increased band intensity was observed in the control plants as compared to stressed plants for example band 14 in the genotypes T 45-1 and ISH 8020B and band 16 in the genotypes T 45-1 and ISH 8020B had greater band intensity at 0.25% salinity but as the level of salinity increased the intensity decreased.

A total of 27 SDS polypeptide bands were identified among different genotypes. However, no definite pattern for appearance or disappearance of bands under saline

condition as compared to control condition was observed. Most of the bands were common in stressed as well as non-stressed plants of the same genotype.

Na^+ concentration in roots of different genotypes increased with increasing salinity. In EC 329299 however, it was higher in control plants as compared to 0.25% and 0.75%. Similarly in EC 318954 also it was almost at par with 0.25 and 0.75%.

The ion estimation among plants in shoot portion revealed that Na^+ concentration increased with increasing salinity in EC 329299, but it was still higher in control plants indicating its exclusion mechanism which restricted transport of Na^+ from roots to shoot portion. In EC 318954 also the Na^+ was more or less equal in control and the plants under stress condition. Variety Wardan also recorded decreased level of Na^+ concentration among plants growing in stress condition. Genotypes like EC 407709, T 45-1, T 5-90I-1 recorded increased level of Na^+ concentration in shoot in the plants growing under stress.

K^+ in root increased with increasing salinity among three genotypes, remained more or less same among two genotypes whereas it decreased with increasing salinity among two genotypes. Decreasing K^+ level with increasing salinity can be considered as indication of resistant nature of the genotypes whereas more or less same level of K^+ in control and stressed plants is indicative of tolerance. As a whole EC 318954 showed minimum K^+ concentration in roots and EC 4017103 and T 5-90I-1 showed maximum K^+ concentration in the roots. Higher K^+ ion in control as compared to stressed plants also indicated that plants preferred to exclude K^+ uptake under stressed condition.

K^+ in shoot increased under stressed condition in two genotypes, remained more or less same among three genotypes whereas it decreased with increasing salinity among four genotypes. As a whole in the shoot also EC 318954 showed minimum K^+ concentration and EC 4017103 and T 5-90I-1 showed maximum K^+ concentration. Higher K^+ ion in control as compared to stressed plants also indicated that plants preferred to exclude K^+ uptake under stressed condition.

Under the experimental condition, the Saidi genotype EC 329299 showed better tolerance mechanism to increasing levels of NaCl treatment as the roots had high K^+ content over the whole salinity range (as compared with control). The root Na^+: K^+ ratio was also low or almost equal to control. This genotype had low content of Na^+ in the shoot region. Thus, low Na^+ in the shoot and high K^+ in the root may be regarded as the mechanism adopted to cope with salinity.

In the Fahli genotype EC 318954 the Na^+: K^+ ratio in the roots under salinity treatments declined as compared to control. Thus, the high uptake of K^+ ions in the roots and low

Na^+: K^+ ratio is the mechanism similar to certain halophytes to cope with increased salinity conditions. Thus, EC 329299 and EC 318954 had similar mechanisms developed for the translocation of excess salts from the shoot region to the roots.

The genotypes T 5-90I-1, EC 4017103 and ISH 8020B had high K^+ content in the shoot across salinity treatments as compared to control, indicating their sensitivity and low tolerance level to salinity conditions due to their failure to exclude Na^+ from the actively growing tissue. These genotypes had high Na^+: K^+ ratio in the shoot indicating their low levels of tolerance to saline conditions and their inability to regulate and control ion transport.

Individual plants differed in their capacity to regulate and control ion transport and accumulation. Thus to improve salt tolerance in *T. alexandrinum* efforts can be made for selecting plants with low Na^+ in the shoot and high K^+ in the roots.

Effect of secondary salinization

Five genotypes were evaluated in sand culture conditions up to 60 days of growth under secondary salinization. Genotype ISH 8020B attained maximum height at 0.50% salinity whereas EC 407709 attained maximum height at 0.75% and 1.0% salinity.

In majority cases the number of leaves was maximum under control condition which gradually decreased with increasing salinity in all the genotypes. Leaf length was also maximum in control condition and was least affected in EC 407709. Under stressed condition at 0.50% and 0.75% salinity ISB 8020 B and EC 407709 showed large leaves. Leaf width was not much affected under saline condition. The differences in number of leaves in the plants growing at different salinity levels were found to be highly significant. Differences for leaf length in the genotypes under different salinity treatments found to be highly significant. Biomass of the plants gradually decreased with increasing salinity. ISH 8020B yielded maximum at 0.50% salinity. However, at 0.75% salinity ISH 8020 B and EC 407709 yielded more than the other genotypes. At 1.0% salinity also EC 407709 yielded maximum. Trend for shoot and root biomass when considered separately also showed the same trend. Analysis of variance revealed that the difference for root and shoot biomass under different salinity levels was highly significant.

The SOD banding pattern in 60 day old plants did not show any specific band appearing or disappearing in saline condition in genotypes EC 329299 and EC 318954. However, SOD band 2 was observed only in plants growing in stressed condition in plants of the other three genotypes i.e. T 45-1, EC 407709 and ISH 8020B. The band 1 in the

genotypes EC 329299 and EC 407709 had greater intensity in all the three salinity treatments as compared to control. Band 3 and 4 had greater intensity at higher salinity in the genotype T 45-1 and ISH 8020B. Thus the bands 1, 2 and 3 had significance for salt tolerance against secondary salinization.

Esterase band 10 in EC 329299, band 6 and 10 in ISH 8020B and band 4 in EC 318954 and T 45-1 were specific to high salinity conditions only. Intensity of band 8 increased under saline conditions as compared to control similarly band 9 in the genotypes EC 329299, EC 318954 and EC 407709 had increased intensity under salt stress.

Native protein Band 9 and 11 in genotype EC 329299 and band 4 and 16 in EC 318954 were salinity specific. Band 5 and 8 were present in stressed plants only in genotype EC 407709 similarly band 1, 4 and 14 were present only in stressed plants in the genotype ISH 8020B and T 45-1 in addition to band 4 in ISH 8020B. Intensity of band 10 in the genotype EC 329299, EC 318954 and EC 407709 increased under saline conditions whereas in the genotype T 45-1 it remained more or less same. Band No. 12 in the genotypes EC 329299, EC 407709 and ISH 8020B had greater intensity in the salinity treatments. Band 13 in the genotypes EC 329299, T 45-1, EC 407709 and ISH 8020B had increased intensity in the salinity treatments as compared to control. Band 14 also had higher intensity in salinity conditions in the genotypes ISH 8020B, EC 407709, T 45-1. Novel proteins bands identified were Band 8 (Rm 0.36), 10 (0.48) and 11 (0.51) in Native PAGE which appeared in saline condition. These results clearly indicated that certain bands appeared under stress conditions. High molecular weight bands 1, 4 and 5 appeared in some genotypes whereas in others low molecular weight bands 9, 11, 14 and 16 appeared under stress. The genotypes under study represented a diverse group and it is quite likely that different genotypes have different proteins synthesized as a defense mechanism.

In the present investigation some proteins showed higher concentrations in stress condition whereas some disappeared. Genotypic response was also different. Differential response of genotypes to increased salinity owing to different mechanism of salt tolerance is also reported in crops like lentil wherein leaf soluble proteins decreased due to salt stress in all lines, irrespective of their salt tolerance.

SDS gel electrophoresis revealed 23 protein bands ranging between 205 Kd to 20 Kd of which 11 bands were monomorphic. Polymorphism was observed for 12 bands (i.e. band no. 1, 3, 4, 5, 8, 9, 10, 16, 17, 19, 20, 21) which accounted for genotypic variation as well

as variation between stressed and non-stressed plants. Most of the bands were represented commonly in stressed and non-stressed plants of the same genotype; however, a few bands appeared in some genotypes under saline condition only such as Band No.16, 19 and 20 in EC 329299, band 9, 16, 17, 20, and 21 in EC 318954, band 8 and 19 in T 45-1 and band 16 in ISH 8020B were salinity specific. Thus, band 16 and 19 (29Kd) appeared to be more salinity specific and seems very near to the 26Kd protein 'osmotin' characterized to be salt induced protein in tobacco.

***In vitro* callusing response**

Hypocotyl explants at low salinity, petiole and hypocotyl explants at moderate salinity and petiole explants at high salinity responded well. In all the genotypes calli derived from different explants developed at various salinity levels responded positively to increased salinity treatment even after second subculture. Callus proliferation was good in SEIM, LSe and LSP3 media also. Prolonging the selection process *in vitro* in rice has been reported to improve the likelihood of regenerating plants with improved salt tolerance.

***In vitro* embryo culture response**

Fertilized flowers of the three ecotypes of *Trifolium* i.e., EC 329299 (Saidi), EC 318954 (Fahli) and Wardan (Mescavi) were brought to laboratory, embryo at cotyledonary stage excised, surface sterilized and inoculated on MS basal media supplemented with 0.3% Kinetin and further supplemented with 0.25%, 0.50% and 0.75% NaCl.

In vitro germination of embryo of the three genotypes ranged between 69.5 to 85.7% in control whereas its response under 0.25% salinity was maximum in Wardan wherein 83.3% embryos germinated as compared to 58.6% in Fahli and Saidi. At higher salinity also maximum germination was noticed in Wardan i.e. 27.3% at 0.75% as compared to 12% in Fahli and Saidi. .

Mortality under control condition ranged from 13.8 to 18.2% among three genotypes. However, high degree of mortality was observed in Fahli and Saidi genotypes under stressed condition. Mortality in these genotypes was up to 100% at 0.75%. In case of Wardan mortality was quite less as compared to other two genotypes at 0.25%, 0.50% and 0.75% salinity 32, 59.1 and 66.7% of the plants degenerated. These results indicated that tolerance against salt stress in embryo development is much higher in Mescavi ecotype as compared to Fahli and Saidi.

Molecular characterization of selected genotypes

RAPD study was carried out using 30 decamer Random primers (Operon Technologies, Inc.) of which 7 did not react while the rest 23 primers generated one or more bands. Primer N-20 produced maximum 14 bands whereas primer OPR-06 and OPF-6 produced minimum 6 bands. In total, 216 bands were generated, of which 71 were polymorphic and 145 monomorphic.

Cluster analysis revealed 82 to 92% similarity among the eight genotypes. Fahli genotype EC 318954 showed 92.27% similarity with Saidi genotype EC 329299. Mescavi genotypes along with exotic, indigenous and tetraploids showed 83.74% similarity. Group of three genotypes T 45-1, EC 4017103 and T 5-90I-1 showed 88.42% similarity. Genotype ISH 8020B which is a cross between *T. alexandrinum* with *T. apertum* showed least similarity (82.69%) with other genotypes. RAPD bands no. 3, 4, 5, 6 and 8 (AB-10), 4 (V-02) and 1 (AB-5 and B-14) were specific to genotypes EC 318954 and EC 329299.

Identification of Genotypes

Identification of genotypes for salt tolerance was done on two criteria i.e. i) the best performing genotypes based on absolute values of traits under observation ii) genotypes showing least reduction under saline condition with respect to control as these genotypes can be good source of resistance genes for different traits.

On the basis of the percent reduction in germination over control genotype such as ISH 34/11, EC 407709 and Penta 99-1 were tolerant for germination at different salinity levels. The genotypes Penta 99, EC 329299 and EC 318954 were found to be tolerant upto 0.50% salinity level but at higher salinity level i.e. 0.75% these recorded more than 50% reduction in germination. For shoot growth among 20 days old seedling, genotypes Penta 99, ISH 34/49 and EC 407709 were most tolerant to varying levels of salinity. For root growth genotypes EC 407709, ISH 34/49, Penta 99 and EC 400977 were comparatively tolerant to other genotypes at varying salinity levels. EC 407709, EC 400977, EC 401711 and ISH 32/8/1 had minimum reduction in number of leaves at all salinity levels indicating their relative tolerance compared to the other genotypes. Genotypes ISH 34/49, Penta 99-1, Penta 99 and T 9-90FM were least affected due to varying levels of salinity for biomass.

On the basis of percent reduction over control in 45 day old plants also genotypes were identified. For shoot length, at 0.25% salinity ISH 5050Y had the least reduction in shoot length (2.5%) over control whereas ISH 32/34/1, T 5-90I-1, ISH 34/49 and T 5-90-I

recorded around 25% reduction. At 0.50% salinity T 5-90-I, ISH 34/49, T 5-90I-1 and T 9-90FM recorded minimum (50%) reduction in shoot length over control whereas the other genotypes recorded 70 to 90% reduction in shoot length. At 0.75% salinity ISH 5050B had the least (60%) reduction, EC 407709 and ISH 32/34/1 had 65% reduction whereas the other genotypes recorded 75 to 90% reduction in shoot length. EC 329299 and EC 318954 had low reduction in root length upto 0.50% salinity whereas EC 407709 had the least reduction at higher salinity as compared to other genotypes. Genotypes EC 329299, EC 318954, Raj Bundi and ISH 32/34/1 recorded less reduction in number of leaves upto 0.50% salinity whereas EC 407709 recorded least reduction at higher level of salinity. For biomass genotypes EC 407709, ISH 34/41 and ISH 32/34/1 had almost minimum reduction in biomass yield at all salinity levels (around 50%) indicating less sensitivity to increasing salinity. Genotype ISH 34/49, Raj Bundi and T 9-90FM had low reduction upto 0.50% salinity. Thus these genotypes can be source of genes of resistance for different traits.

On the basis of performance following genotypes figured among top five rankers in 20 days and 45 days observation:

Germination: ISH 34/11, EC 407709, EC 318954, EC 329299, ISH 34/49

Shoot length: EC 407709, EC 329299, EC 401711

Root growth: EC 407709, EC 318954, EC 329299, ISH 8020B

No. of leaves: EC 407709, EC 329299, ISH 34/8B

Weight of plants: T 9-90FM, EC 407709, EC 329299, EC 318954, T 5-90-I, ISH 34/8B

On the basis of total score of various traits at different salinity levels EC 407709, EC 318954 and EC 329299 were found top ranking.

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